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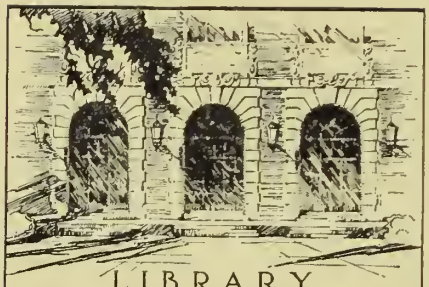

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UPTAKE OF SOLUBLE PHOSPHATE BY ACTIVATED SLUDGE

By

MILLARD WAYNE HALL

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UPTAKE OF SOLUBLE PHOSPHATE BY ACTIVATED SLUDGE

by

Millard Wayne Hall

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ABSTRACT

A laboratory investigation was performed, using bench scale activated sludge units of the fill and draw type, to study the removal kinetics and mechanisms responsible for the removal of soluble phosphates by activated sludge systems.

This investigation has shown that a heterogenous population of microorganisms characteristic of activated sludge may be utilized to attain significant reductions in the soluble phosphate content of biologically treatable wastewaters. The conditions necessary for this occurrence were shown to include the presence of an active microbial population, sufficient quantities of both organic and inorganic nutrients, an aerobic environment, and an adequate period of contact between the microorganisms and the waste being treated. It was also shown that essentially all of the phosphate removed from solution under these conditions could be traced to the microbial cells composing the activated sludge. It was concluded that the uptake of soluble phosphate in activated sludge mixed liquors is related to the metabolic activity of the aerobic microorganisms present in the system.

Several other mechanisms which had been proposed as explanations for the observed disappearance of soluble phosphate from activated sludge mixed liquors were examined. None of these mechanisms was found to be of significance in reducing the soluble phosphate content of the mixed liquors used in this investigation.

A number of variables which were purported to affect the uptake of soluble phosphate in activated sludge mixed liquors were also studied.

These included mixed liquor suspended solids concentration, substrate concentration, dissolved oxygen concentration, degree of mixing, pH, and soluble phosphate concentration. It was shown that, within the limits studied, substrate concentration, dissolved oxygen concentration, pH, and soluble phosphate concentration exerted a pronounced influence on the rate and magnitude of soluble phosphate uptake. On the other hand, mixed liquor suspended solids concentration and degree of mixing exerted no observable effect on this uptake.

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TABLE OF CONTENTS

	Page
ACKNOWLEDGMENTS	iii
LIST OF TABLES	vi
LIST OF FIGURES	vii
I. INTRODUCTION	1
II. LITERATURE REVIEW	4
III. PURPOSE AND SCOPE OF THE INVESTIGATION	15
IV. MATERIALS AND METHODS	16
A. Experimental Equipment	16
B. Bacterial Cultures	16
C. Analytical Procedures	19
1. Mixed Liquor Suspended Solids	19
2. Chemical Oxygen Demand	19
3. Dissolved Oxygen	20
4. pH	20
5. Phosphorus Concentration	21
a. Soluble Orthophosphate	22
b. Soluble Total Inorganic Phosphate	23
c. Total Phosphorus	25
6. Fractionation Procedure	30
V. RESULTS AND DISCUSSION	33
A. Preliminary	33
B. Phosphorus Balance in Activated Sludge	35
1. Evaluation of Suggested Mechanisms of Soluble Phosphorus Uptake by Activated Sludge	38

Page

2. Cellular Phosphorus Balance 45

C. Influence of Mixed Liquor Suspended Solids Concentration 52

D. Influence of Initial Substrate Concentration . 62

E. Influence of Dissolved Oxygen Concentration . . 73

F. Influence of pH 84

G. Influence of Initial Soluble Phosphorus Concentration 85

VI. ENGINEERING SIGNIFICANCE 99

VII. SUMMARY AND CONCLUSIONS 103

VIII. SUGGESTIONS FOR FUTURE WORK 108

REFERENCES 109

APPENDIX A 114

VITA 115

LIST OF TABLES

Table		Page
1	SUBSTRATE AND NUTRIENT CONCENTRATION AND OTHER OPERATING PARAMETERS OF STOCK FILL AND DRAW ACTIVATED SLUDGE UNIT	18
2	DESCRIPTION OF EXPERIMENTAL UNITS USED FOR PHOSPHORUS BALANCE IN ACTIVATED SLUDGE UNITS	39
3	CELL FRACTIONATION STUDIES, PHOSPHORUS BALANCE IN ACTIVATED SLUDGE	47
4	CELL FRACTIONATION STUDIES, DISTRIBUTION OF INSOLUBLE PHOSPHORUS	50
5	OPERATING PARAMETERS: MLSS CONCENTRATION STUDIES	55
6	UPTAKE OF SOLUBLE PHOSPHORUS AND COD: MLSS CONCENTRATION STUDIES	61
7	SUMMARY OF RECOMMENDED BOD:P RATIOS FOR TREATMENT OF WASTES BY ACTIVATED SLUDGE	63
8	SUBSTRATE AND NUTRIENT CONCENTRATION AND OTHER OPERATING PARAMETERS: INITIAL SUBSTRATE CONCENTRATION STUDIES	65
9	CELL FRACTIONATION STUDIES, INFLUENCE OF INITIAL SOLUBLE PHOSPHORUS CONCENTRATION ON SOLUBLE PHOSPHORUS UPTAKE	93
10	CELL FRACTIONATION STUDIES, DISTRIBUTION OF CELLULARLY ASSOCIATED PHOSPHORUS IN HIGH AND LOW SOP_i SYSTEMS	96

LIST OF FIGURES

Figure		Page
1	SOLUBLE ORTHOPHOSPHATE CONCENTRATION IN MIXED LIQUOR DURING AERATION, BALTIMORE SEWAGE TREATMENT PLANT, (22)	7
2	SOLUBLE PHOSPHORUS REMOVAL FROM SEWAGE BY ACTIVATED SLUDGE, (24)	9
3	CALIBRATION CURVE FOR THE DETERMINATION OF SOLUBLE ORTHOPHOSPHATE	24
4	CALIBRATION CURVE FOR THE DETERMINATION OF TOTAL INORGANIC PHOSPHATE	26
5	CALIBRATION CURVE FOR THE DETERMINATION OF TOTAL PHOSPHORUS	29
6	VARIATION IN SOLUBLE PHOSPHORUS, SOLUBLE COD AND MLSS DURING AERATION OF ACTIVATED SLUDGE FROM STOCK UNIT	34
7	TOTAL SOLUBLE PHOSPHORUS, SOLUBLE ORTHOPHOSPHATE, AND TOTAL GROSS PHOSPHORUS DURING AERATION OF ACTIVATED SLUDGE FROM STOCK UNIT	41
8	SOLUBLE PHOSPHORUS UPTAKE IN ACTIVATED SLUDGE AND BLANK SYSTEMS DURING AERATION	44
9	SOLUBLE PHOSPHORUS UPTAKE, CELL FRACTIONATION STUDIES	49
10	VARIATION IN SOLUBLE COD DURING AERATION OF ACTIVATED SLUDGE: MLSS STUDIES	56
11	VARIATION IN MLSS DURING AERATION OF ACTIVATED SLUDGE: MLSS STUDIES	57
12	SOLUBLE PHOSPHORUS UPTAKE AS A FUNCTION OF MLSS CONCENTRATION	59
13	INFLUENCE OF INITIAL SUBSTRATE CONCENTRATION ON UPTAKE OF SOLUBLE PHOSPHORUS	66
14	PHOSPHORUS UPTAKE AS A FUNCTION OF INITIAL SUBSTRATE CONCENTRATION	67

Figure		Page
15	SOLUBLE PHOSPHORUS UPTAKE AS A FUNCTION OF COD UPTAKE	69
16	SOLUBLE PHOSPHORUS UPTAKE AS A FUNCTION OF MLSS SYNTHESIS	71
17	UPTAKE OF SOLUBLE PHOSPHORUS AT VARIOUS INITIAL SUBSTRATE CONCENTRATION - CONSTANT INITIAL SOLUBLE PHOSPHORUS CONCENTRATION, 45 mg/l as P_2O_5	74
18	SOLUBLE PHOSPHORUS UPTAKE, SYSTEM RESPONSE TO CONTINUOUS AERATION, MIXING WITH N_2 AND QUIET SETTLING	77
19	SOLUBLE PHOSPHORUS UPTAKE, SYSTEM RESPONSE TO AERATION RATE	79
20	PHOSPHORUS UPTAKE AS INFLUENCED BY DISSOLVED OXYGEN	81
21	SOLUBLE PHOSPHORUS UPTAKE AS A FUNCTION OF DISSOLVED OXYGEN CONCENTRATION	83
22	SOLUBLE PHOSPHORUS UPTAKE AS A FUNCTION OF pH . .	86
23	SOLUBLE PHOSPHORUS UPTAKE AS A FUNCTION OF INITIAL SOLUBLE PHOSPHORUS CONCENTRATION	88
24	VARIATION IN SOLUBLE COD AND MLSS CONCENTRATION DURING AERATION OF ACTIVATED SLUDGE: INITIAL SOLUBLE PHOSPHORUS CONCENTRATION STUDY	90
25	INFLUENCE OF INITIAL SOLUBLE PHOSPHORUS CONCENTRATION ON TOTAL 6 HOUR UPTAKE OF SOLUBLE PHOSPHORUS BY ACTIVATED SLUDGE SYSTEMS OPERATED AT DIFFERENT LOADING RATES	92
26	PHOSPHORUS CONCENTRATION OF NUCLEIC ACIDS AND METABOLIC INTERMEDIATES -- RESPONSE TO INITIAL SOLUBLE PHOSPHORUS CONCENTRATION	95

I. INTRODUCTION

During recent years an increased concern has developed with regard to the excessive enrichment of surface waters by inorganic nutrients contained in the effluents from municipal and industrial wastewater treatment facilities. The traditional objectives of sewage treatment, i.e., the reduction of the biochemical oxygen demand, and the removal of particulate matter, are no longer always adequate to protect surface water supplies from pollution. The biological oxidation of sewage liberates the mineral constituents of the organic matter being treated. These newly released minerals, along with the inorganic nutrients already present in the influent sewage, are, for the most part, discharged in the effluents from treatment facilities.

As the volume of wastewater has grown, the effect of these dissolved minerals in establishing an undesirable proliferation of algae and aquatic plants within receiving streams has become increasingly evident. A number of investigators (1-4) have reported that the dissolved inorganic nutrients of greatest significance in this respect are the compounds of nitrogen and phosphorus, particularly phosphorus.

At the same time that the growth in wastewater volume was bringing about the intensification of this problem, the expanded use of phosphates in agricultural fertilizers, in synthetic detergents, and in a variety of industrial processes, greatly increased the concentration of phosphorus compounds in the aqueous environment (5). This has had the effect of further aggravating an already serious problem.

The importance of phosphorus as a limiting factor in controlling obnoxious aquatic growth has been emphasized by the investigations of Fogg and Wolfe (6), Buljan (7), and others (8, 9). These investigators have demonstrated that certain forms of blue-green algae, among them Anabaena, Anacystis, Aphanizomenon, Calothrix, and Nostoc, are capable of fixing atmospheric nitrogen, thereby augmenting the supply of usable nitrogen in their environment. In many instances, therefore, algae may be able to utilize all available phosphorus, making phosphorus the limiting factor in the development of algae blooms. For this reason, it is generally held that the removal of phosphorus from wastewaters should be helpful in the control of obnoxious algae blooms.

Because of the foregoing considerations, interest is currently running very high in methods for reducing the phosphorus concentration of secondary effluent. A Presidential Advisory Committee, formed in 1965, has considered this problem. Department of the Interior Secretary, Stewart L. Udall, has recently indicated that the cities of Cleveland, Ohio and Detroit, Michigan will be required to remove phosphate from their sewage effluents to protect Lake Erie from excessive algae growths (10). The State of Virginia currently requires phosphate removal from sewage effluents discharged into the Potomac River in the vicinity of metropolitan Washington, D. C. (10).

In response to this interest, numerous treatment processes have been proposed as means of dealing with the problem. All of these proposals have involved tertiary treatment of one form or another. For a variety of reasons, principally economics, none of these proposed methods has been widely accepted. This suggests, therefore, that a different approach to this problem is needed.

It seems rather strange that so little interest has been shown in evaluating the phosphorus removing capabilities of existing wastewater treatment facilities. As mentioned earlier, most published data indicate that very little phosphorus is being removed in conventionally operated secondary treatment plants. Certain investigators, however, have demonstrated that activated sludge treatment systems can, when operated under certain conditions, remove significant amounts of phosphorus from the waste stream. The Sewage Commission of the City of Milwaukee, for instance, reports phosphorus removals of 60 percent or better in their two plants (11). Levin and Shapiro (12), investigating phosphorus removal in the District of Columbia Sewage Treatment Plant, reported that a significant increase in phosphorus removal could be effected by the simple expedient of increasing the aeration rate. More recently, it has been reported that operational changes in two activated sludge plants in San Antonio, Texas have increased phosphorus removal from "typically low" values to "over 90 percent" (13). The nature of the operational modifications which are responsible for the improved phosphorus removal efficiency has not yet been reported.

These recent findings point to the possibility of significant reductions in secondary effluent phosphorus concentration through the proper utilization of existing waste treatment facilities, and indicate the need for continued research in this area.

The investigation reported herein was undertaken to delineate some of the factors which contribute to the uptake of soluble phosphorus by activated sludge treatment systems. It is believed that the information obtained from this study will be useful in improving the phosphorus removal efficiencies of activated sludge systems, and will contribute fundamental knowledge regarding the uptake of phosphorus by activated sludge mixed liquor.

II. LITERATURE REVIEW

The role of phosphorus as a key element in man-induced eutrophication of surface waters, and the numerous proposals advanced as means for reducing the concentration of this element in the aqueous environment have been reviewed in great detail by several authors (14-18). It is felt that nothing is to be gained from a reiteration of their views in these pages. Consequently, the purpose of the following review is to cover only those past efforts that are in keeping with the purpose and scope of this investigation. Specifically, only those references that contain pertinent information related to the utilization of the activated sludge process for reducing the phosphorus concentration of secondary effluents have been selected for review here.

Because of economic considerations, sewage treatment has always relied heavily upon naturally occurring biological processes. It seems reasonable, therefore, to investigate the possibility of using a biological method for removing phosphorus from sewage effluents. Surprisingly, very little attention has been directed towards this possibility.

Wuhrmann (19) investigated the elimination of phosphorus, calcium, and nitrogen from sewage effluent through metabolic uptake by heterotrophic microorganisms. He explored the use of the bacteria present in sewage and sludge, and concluded that the carbon:nitrogen:phosphorus (C:N:P) ratio in domestic sewage demonstrates a large deficit in organic carbon. He concluded that insufficient usable carbon was present in domestic sewage to permit significant reduction in dissolved phosphorus. The activated sludge treatment plant of the City of Zurich was cited as achieving a 25 percent reduction in dissolved phosphate, and the implication was that additional phosphorus uptake was limited by carbohydrate deficiencies.

Bogan (3) also studied the possibility of using the microorganisms which constitute activated sludge to take up phosphate, and dismissed it on the grounds that, "Ordinary domestic sewage does not provide a balanced diet for activated sludge, being deficient in carbon and nitrogen with respect to the amount of phosphorus normally present" (3).

Stumm and Morgan (20) compared the relative composition of domestic sewage with the mean stoichiometric relation between carbon, nitrogen, and phosphorus in bacterial organisms. They concluded that present day aerobic biological treatment is not capable of eliminating more than 20 to 50 percent of the nitrogenous and phosphatic constituents of domestic sewage, because of the limited availability of organic carbon in the sewage. They also stated that the C:N:P ratio of the waste is a decisive factor in the elimination efficiency of inorganic nutrients by biological treatment. Thus, the treatment of wastes with high ratios of assimilable carbon to inorganic nutrients, such as sugar wastes, generally leads to higher nutrient removal efficiencies.

This view was presented earlier by Sawyer (21), who demonstrated that the periodical addition of glucose to activated sludge, over a period of several days, would finally produce an effluent free of inorganic phosphorus. He stated that such a process might find limited application in the treatment of certain wastes, but pointed out the adverse economics involved in such a scheme.

Although all of these researchers offer evidence opposing the possibility of significant reduction in effluent phosphorus concentration through the use of the activated sludge process, other investigators have reported data which do not necessarily fit the carbohydrate, i.e., available carbon, limitation theory.

Alarcon (22), studying phosphate fractions present in the various stages of the Baltimore, Maryland sewage treatment plant, found that the dissolved phosphate content of the effluent from the activated sludge process varied widely from time to time. At one sampling, the effluent from the activated sludge process contained only one-tenth the dissolved phosphate present in the effluent from the trickling filter. At this plant a portion of the flow goes to the activated sludge system, with the remainder being diverted to the trickling filters, so that the character of the raw waste entering both systems would have been essentially the same. A second sampling failed to show any significant difference between the respective dissolved phosphate concentration of the two effluents. Alarcon suspected that insufficient aeration in the activated sludge process might have been the cause of the high phosphate content in the aeration tank effluent of the second sampling. Rudolfs (23) had earlier reported that phosphorus was released from sludge which was allowed to become anaerobic.

Accordingly, Alarcon (22) took samples of the activated sludge mixed liquor into the laboratory for closer observation under conditions of vigorous aeration. Aliquots of the vigorously aerated samples were withdrawn periodically, and filtered through a membrane filter to remove all particulates, including bacteria. The orthophosphate content of the filtrate was then determined. The results of this experiment are shown in Figure 1. Initial uptake was quite pronounced, and was followed by uptake at a decreasing rate which resulted in almost total removal of the dissolved orthophosphate by the sixth hour of aeration. Alarcon concluded that the decrease in the orthophosphate content of the filtrate

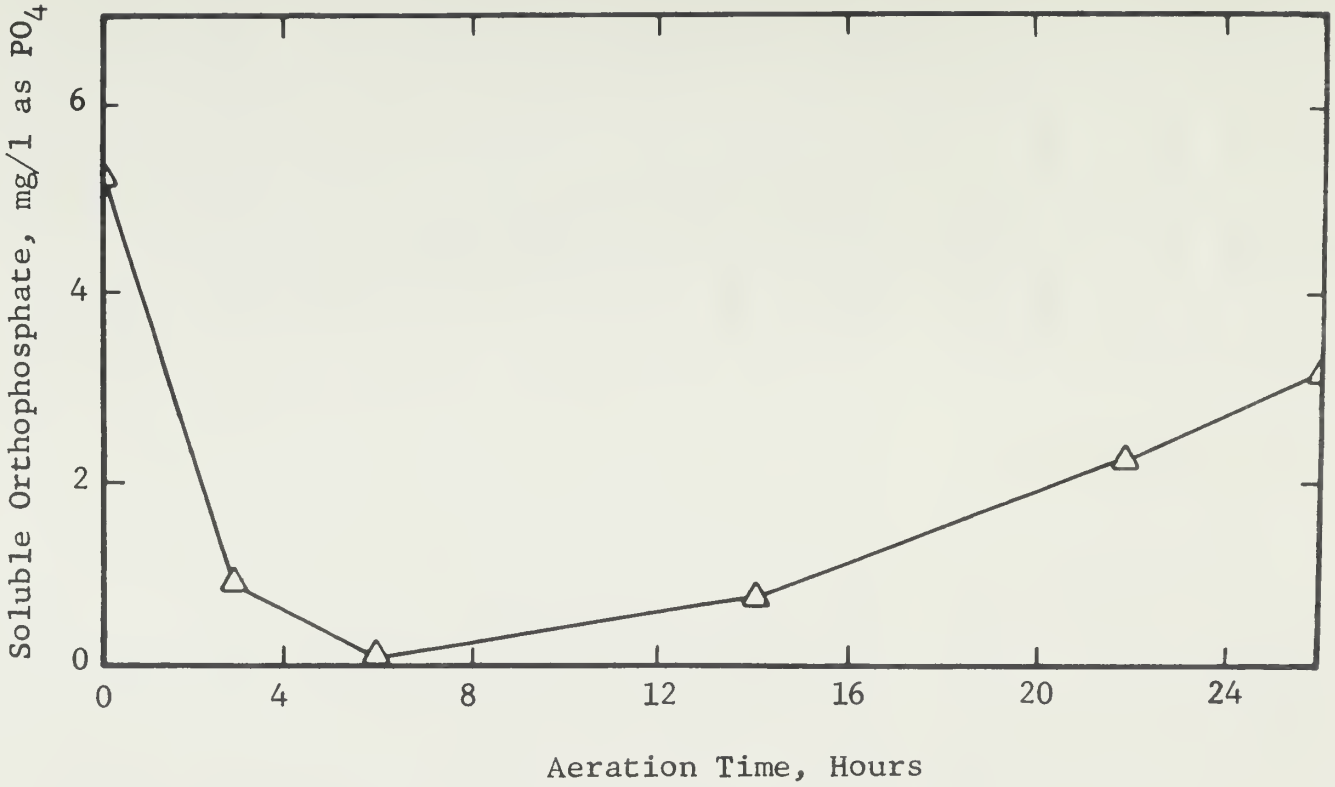

FIGURE 1 SOLUBLE ORTHOPHOSPHATE CONCENTRATION IN MIXED LIQUOR DURING AERATION, BALTIMORE SEWAGE TREATMENT PLANT, (22).

was the result of the uptake of dissolved orthophosphate by the activated sludge. His data also indicate that orthophosphate was apparently secreted by, or leaked out of, the sludge solids after the sixth hour of aeration. By the 26th hour of aeration the orthophosphate content of the filtrate had returned to about 60 percent of its original value.

Attempting to demonstrate the biological nature of this "uptake" of dissolved orthophosphate, Alarcon added mercuric chloride to aerating mixed liquor, and followed the dissolved orthophosphate content of the filtrate. The uptake of orthophosphate in the presence of mercuric chloride was only 10 percent of the initial dissolved orthophosphate content of the system.

Similar investigations, presumably unknown to Alarcon, had been performed in 1959 by Srinath et al. (24) in India, where they were attempting to grow rice in "hanging gardens" in the effluent end of an activated sludge tank which provided a four-hour aeration period. Srinath and his co-workers observed that, although the influent raw sewage contained "a considerable amount" of water-soluble phosphorus, the rice plants suffered from a serious phosphorus deficiency. These investigators examined the process in much the same manner as Alarcon, by aerating volumes of mixed liquor and analyzing for dissolved phosphorus in the settled supernatant. Figure 2 presents the results obtained. As in Alarcon's later work, these results showed a rapid initial decrease in supernatant dissolved phosphorus during the first 45 minutes of aeration, followed by a declining rate of disappearance which resulted in almost complete removal of the dissolved phosphorus by the sixth hour.

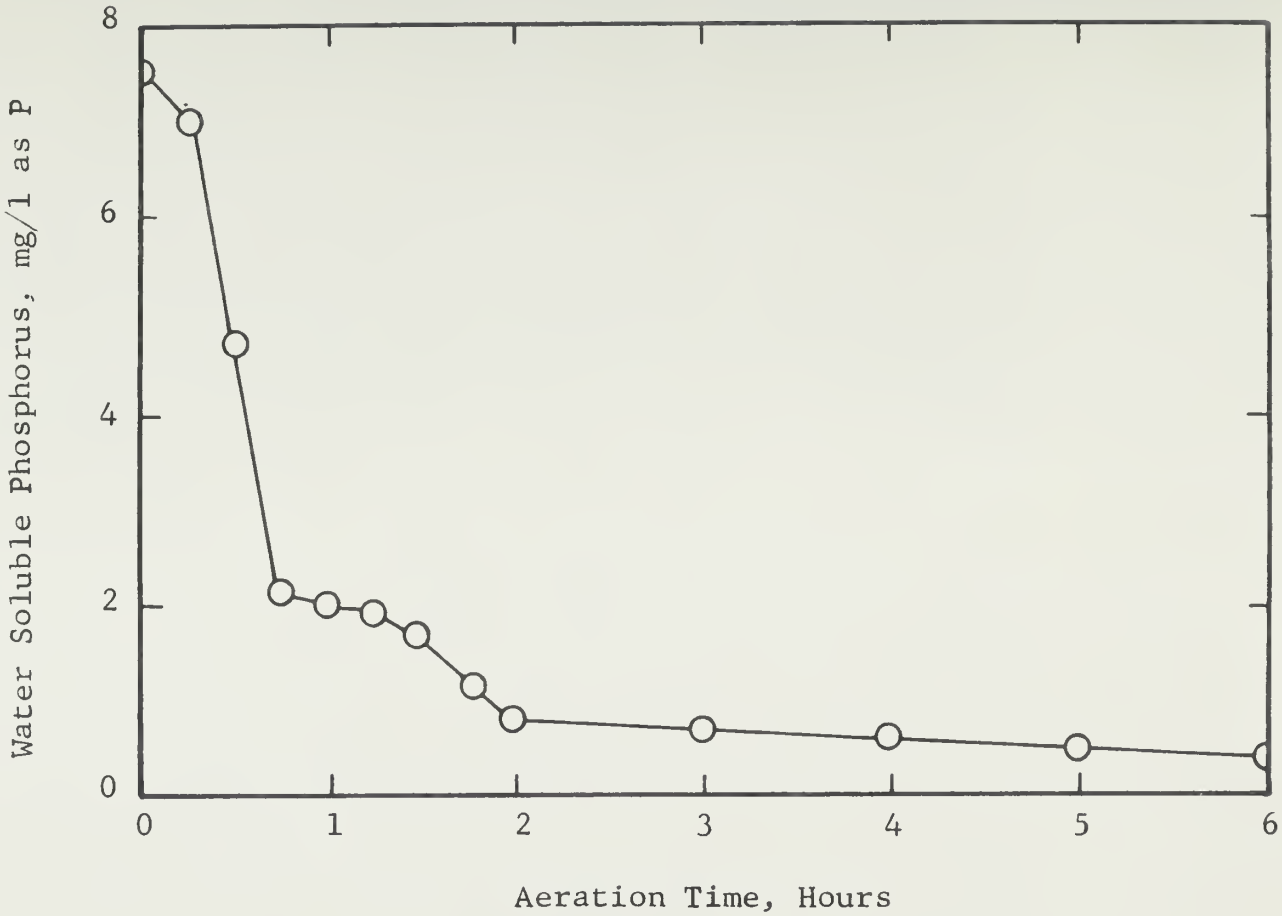

FIGURE 2 SOLUBLE PHOSPHORUS REMOVAL FROM SEWAGE BY ACTIVATED SLUDGE, (24).

Srinath and his co-workers also determined the three minute permanganate value of the raw sewage and settled supernatants, a measure of the readily oxidizable carbonaceous material present in the waste. They reported that a reduction in the dissolved phosphorus content of the waste occurred in conjunction with a reduction in the three minute permanganate value. This seemed to suggest that the observed reduction in soluble phosphorus might somehow be associated with microbial metabolism.

These investigators attempted to demonstrate the biological nature of the phosphorus uptake by the addition of mercuric chloride, as well as by heating the sludge to 40°C for ten minutes prior to the mixing of the sludge with the raw waste. In both cases, phosphorus uptake was adversely affected.

Srinath et al. also deomstrated the effect of the solids content of the mixed liquor on phosphorus uptake. They reported that maximum uptake was produced by "20 to 30 percent sludge," i.e., 20 to 30 ml of return sludge per 100 ml of mixed liquor, which achieved water soluble phosphorus reduction, in the settled supernatant, in the 90 percent range after six hours aeration. Aerated raw sewage, i.e., zero percent sludge, took up practically no phosphorus during the same period.

In 1962, Feng (25) reported on the results of investigations into the usefulness of the soluble orthophosphate concentration of activated sludge mixed liquor as an index of the biological activity of the process. He demonstrated the ability of activated sludge to remove much of the orthophosphate from solution. His results indicated that this removal was dependent upon temperature, aeration rate, and the mixed liquor suspended solids (MLSS) concentration of the system.

Levin (14), in 1963, reported on further studies of this phenomenon, which he had conducted at the District of Columbia Sewage Treatment Plant. In laboratory scale experiments, Levin found that a small amount of return sludge in raw sewage dramatically improved soluble orthophosphate uptake over that obtainable in raw sewage alone. He was able to show that as much as 80 percent of the dissolved orthophosphate contained in a mixed liquor solution composed of 30 percent return sludge and 70 percent raw sewage, by volume, was "...taken up by the sludge organisms with no addition of substrate." He also announced the demonstration of a "luxury" uptake of soluble orthophosphate, i.e., "...uptake in the absence of growth." He concluded that "...rate of aeration, pH, and return sludge..." are highly important variables affecting "metabolic uptake" of orthophosphate by activated sludge mixed liquors during aeration.

Rudolfs (23), in 1947, reported a release of soluble phosphorus from sludge which had been permitted to become anaerobic. This has been mentioned previously in connection with Alarcon's (22) hypothesis concerning the difference in orthophosphate content of effluents from trickling filter and activated sludge systems. The study made by Levin (14) confirmed that orthophosphate leaked from the particulate matter in the mixed liquor when the activated sludge was permitted to settle with no aeration. Levin also demonstrated that a similar leakage, or secretion, of orthophosphate occurred at pH's of 5.0 and 6.0 even though the mixed liquor was being aerated.

Campbell (26), reported that return sludge, kept under anaerobic conditions at room temperature for four hours leaked phosphate from the sludge solids into the liquid.

In addition to the loss of orthophosphate from the particulate matter back into the liquid environment under adverse conditions of dissolved oxygen and pH, another type of orthophosphate leakage, or possibly secretion, has been reported. Data presented by Sekikawa et al. (27), as well as that reported by other investigators (14, 22), show that soluble orthophosphate is released from MLSS during extended aeration. Sekikawa and his group also reported that the aerobic digestion of solids resulted in the release of soluble orthophosphate.

Irgens and Halvorson (28), on the other hand, while studying the aerobic stabilization of a mixture of activated and raw sewage sludges, found that there was a significant uptake and retention of orthophosphate by the solids in the system. It was concluded that phosphate was being utilized for the synthesis of new microbial protoplasm and retained in the solids.

Vacker, Connell, and Wells (29) recently reported on studies carried out at three activated sludge plants in San Antonio, Texas. They showed up to 90 percent removal of total phosphate in an activated sludge plant where digester liquors were not returned to the system. They concluded that plant loading rate, dissolved oxygen concentration in the aeration tanks, and the degree of return of digester liquors were important parameters in the operation of an activated sludge plant for maximum phosphate removal. Their work also indicated that leaking of phosphate from the solids back into the liquid under anaerobic conditions was not significant once the phosphate uptake was complete and a high dissolved oxygen level had been sustained throughout the end half of the aeration tank. According to other sources (13, 30) wastes at several other plants

around the country have since been treated experimentally in the same manner and, without exception, very high phosphate removal has been achieved.

Levin (31, 32) has obtained a patent on a system for reducing the amount of phosphate in activated sludge sewage treatment plant effluent. The system is based on the ability of activated sludge microorganisms to take up more phosphate than they need, when the aeration rate is three to five times greater than normal. To recover the phosphate, the organisms are removed from the mixed liquor by froth flotation. The sludge is then allowed to stand in an anaerobic tank, where the organisms give up their phosphate. The organism-containing sludge is then recycled to the aeration tank and the phosphate-rich supernatant fluid is treated with lime for the precipitation of tricalcium phosphate.

Alarcon (22), Srinath et al. (24), and others (14, 27) have clearly demonstrated the changing rate of phosphate uptake. Their work suggests that phosphate can be taken up in excess of the limitation that would be imposed by a high C:N:P ratio in cellular tissue. In addition to these suggestive results, there is evidence that bacteria are able, under certain conditions, to store phosphorus.

Harold (33) states that metachromatic granules, sometimes called "volutin," have been observed in microorganisms since 1888. These granules have been found to correspond to deposits of inorganic polyphosphate. They occur in fungi, algae, and bacteria. The function of these deposits is not yet understood (33, 34). However, it is apparent that, if phosphorus can be stored within the cells comprising activated sludge, the C:N:P ratio

of the cells would be variable, and would no longer be a significant theoretical barrier to the possibility of utilizing these cells for reducing the phosphorus content of secondary effluents. In this connection, it is interesting to note that as early as 1955, Greenberg, Klein and Kaufman (35) reported that activated sludge organisms were able to take up more phosphorus than that actually required for satisfactory BOD removal.

III. PURPOSE AND SCOPE OF THE INVESTIGATION

The investigations cited in the previous chapter have contributed a great deal of information with regard to reducing the phosphorus concentration of activated sludge effluents. However, it is evident that a number of questions regarding the process or processes involved in this reduction remain unresolved. Present knowledge concerning the uptake and release of soluble phosphates by activated sludge mixed liquor appears to be insufficient to permit the application of this process as a practical means for controlling the phosphorus content of effluents from existing treatment facilities. Consequently, there is still a definite need to obtain additional information concerning the removal of soluble phosphorus by the activated sludge treatment process.

The present investigation was undertaken to study the removal kinetics and mechanisms responsible for the removal of soluble phosphates in laboratory scale activated sludge systems, under various operating conditions. The variables which reportedly affect the removal kinetics of soluble phosphates in an activated sludge system and which were studied were: MLSS concentration, substrate concentration, dissolved oxygen concentration, pH, degree of mixing, and soluble phosphate concentration. These parameters were selected following an appraisal of the existing literature on the subject.

IV. MATERIALS AND METHODS

A. Experimental Equipment

The activated sludge used in the various phases of this investigation was obtained from laboratory scale activated sludge units of the fill and draw type, which received a synthetic substrate consisting of glucose, yeast extract, and appropriate minerals. These units consisted of 65 by 500 mm Pyrex brand glass test tubes, having a total volume of approximately 1400 ml. The culture volume in each unit was one liter. Each cylinder was covered with a rubber stopper to prevent the loss of liquid through spraying. Air, nitrogen gas, and carbon dioxide were supplied as needed through fritted glass diffusers suspended from rubber tubes passed through holes in the stoppers.

The compressed air which supplied these units was first passed through an oil trap, and then diffused through distilled water so as to saturate it with water vapor. This procedure insured a clean air supply, and also considerably reduced evaporation losses in the units.

Throughout this investigation, these units were operated in a constant temperature water bath at 25°C.

B. Bacterial Cultures

To initiate this investigation, a single activated sludge unit was started, using seed obtained from the waste treatment plant operated by the Urbana-Champaign Sanitary District, Urbana, Illinois. This and all subsequent units were operated as follows. After 23.5 hours of aeration, solid material adhering to the inner surfaces of the unit was resuspended and distilled water was added to compensate for evaporative losses. After mixing, the required amount of mixed liquor was wasted, and aeration was

discontinued for one-half hour to allow the sludge to settle. After settling, the supernatant liquid was drawn down to the 250 ml mark, and the nutrients and substrates were added. The unit was then refilled to the operating level with distilled water and aeration was resumed. When filled to the one-liter operating level, the units had a freeboard of approximately five inches.

The original unit, hereafter designated as the stock unit, was operated so as to contain, immediately after feeding, an MLSS concentration of 1500 mg/l, and a COD concentration of 3000 mg/l. This corresponds to a loading rate (F:M) of 2, i.e., 2 gm COD/gm of MLSS - day. A mixture of glucose and yeast extract in a ratio of 4:1 (as COD) was used as the organic substrate for this and all subsequent units. Additional nitrogen, in the form of ammonium ion, was supplied to maintain a COD:N ratio of approximately 15. Phosphorus, in the form of monobasic potassium phosphate (KH_2PO_4) and dibasic sodium phosphate ($Na_2HOP_4 \cdot 7H_2O$), was supplied to maintain a COD:P ratio of approximately 75:1. Table 1 lists the substrates, nutrients, and other operating parameters used in the maintenance of the stock unit.

In order to insure that this and all other units were functioning properly, measurements of pH and MLSS concentration were made daily.

As they were needed, other units were started, using seed obtained from the stock unit, and acclimated to the various conditions being studied.

As soon as the MLSS concentration stabilized, indicating completed acclimation, experiments were begun. The experimental procedure

TABLE 1

SUBSTRATE AND NUTRIENT CONCENTRATION AND OTHER OPERATING PARAMETERS OF STOCK FILL AND DRAW ACTIVATED SLUDGE UNIT

Compound	Initial Concentration (mg/l except as indicated)
Glucose	2400 (as COD)
Yeast extract	600 (as COD)
NH_4Cl	600
$MgSO_4 \cdot 7H_2O$	250
$FeSO_4 \cdot 7H_2O$	10
$MnSO_4 \cdot H_2O$	10
$CaCl_2 \cdot 2H_2O$	10
KH_2PO_4	68
$Na_2HPO_4 \cdot 7H_2O$	134
Na_2CO_3	2650
Tap water	100 ml/liter
pH	7.5
Waste schedule	50% of mixed liquor/ day
Initial loading rate (F:M)	2
Air flow rate	4 ft^3/(hr)(l)

was initiated by harvesting the waste sludge from the acclimated unit under study. The harvested sludge was allowed to settle, and the supernatant then decanted. Substrate, nutrients, and water were then added so as to prepare an experimental unit, identical in all respects to the acclimated unit, which was sacrificed during the course of the experiment. In this way, the acclimated unit was protected, and could later be used for replicate studies.

C. Analytical Procedures

1. Mixed Liquor Suspended Solids

The determination of mixed liquor suspended solids concentration was made by the membrane filtration method described by Winneberger et al. (36). Membrane filter disks having a pore size of 0.45 microns and a diameter of 47 mm were used for all determinations. The stainless steel membrane filtration apparatus and the filter disks were supplied by the Millipore Filter Corporation, Bedford, Massachusetts.

2. Chemical Oxygen Demand

Chemical oxygen demand (COD) was determined in accordance with Standard Methods (37). The following quantities of reagents and sample were used in this investigation:

20 ml of sample of aliquot diluted to 20 ml

10 ml of 0.25 N potassium dichromate

30 ml of concentrated sulfuric acid containing 22 gm of silver sulfate per nine-pound bottle of acid

0.10 N ferrous ammonium sulfate

0.4 gm mercuric sulfate per sample

The mercuric sulfate addition is recommended for the elimination of chloride interference.

3. Dissolved Oxygen

Measurements of dissolved oxygen concentration were made using a YSI Model 51 Oxygen Meter, supplied by the Yellow Springs Instrument Company, Incorporated, Yellow Springs, Ohio. This instrument was calibrated in air-saturated distilled water at 25°C each time it was used.

4. pH

Practically all of the studies performed to date by other researchers, using laboratory scale activated sludge systems, have utilized phosphate buffer systems for pH control. However, because of the extremely high phosphorus concentration required of such systems, it was felt that they would be unsuitable for use in the present investigation. Consequently, a carbonate buffer system was employed for pH control in this study. This buffer system was prepared as follows. Initially a small volume of a 1.0M solution of sodium carbonate was added to each activated sludge unit. A small quantity of gaseous carbon dioxide was then diffused into the system until an equilibrium pH was established. The pH was then adjusted up or down to its desired value by changing either the amount of sodium carbonate added to the system or by adjusting the flow of carbon dioxide entering the system, or by a combination of these changes.

For several reasons, chiefly economics and ease of operation, the units used in this investigation were operated so as to use a minimum amount of carbon dioxide for a given pH. In other words, pH control was exercised as much as possible by controlling the sodium carbonate concentration of the systems. Additional fine control was obtained, as necessary, by adjustment of the amount of carbon dioxide entering the system.

It was found that a buffer system such as this, though easy to generate, requires somewhat more attention than buffer systems commonly employed for this type of work. However, with a little practice it is not difficult to control the pH to within $\pm$ 0.1 unit of the desired level.

Measurements of pH were made using a Beckman Model 76 Expanded Scale pH Meter with a Beckman 39183 Combination Electrode. This equipment was manufactured by Beckman Instruments, Incorporated, Fullerton, California.

5. Phosphorus Concentration

Phosphorus concentrations were measured as soluble orthophosphate, soluble total inorganic phosphate, total soluble phosphorus, and total gross phosphorus, i.e., total phosphorus in an unfiltered sample. The procedures used for orthophosphate and total inorganic phosphate are modifications of the methods outlined in Standard Methods (37). These modifications were suggested by Dr. David I. Jenkins (38). The procedure used for total phosphorus, both soluble and gross, is a modification of the procedure suggested by Harris and Popat (39). These procedures are outlined below. All reagents except those indicated by an asterisk were prepared according to the 12th Edition of Standard Methods (37). Directions for preparing the noted reagents are given in Appendix A.

The phosphorus data presented in this report are expressed as P_2O_5, except where indicated. This choice was made rather arbitrarily, and is simply a reflection of the author's past experience in presenting such data. In an earlier work (40), dealing with the occurrence and persistence of phosphate compounds in activated sludge, phosphate concentrations were determined as recommended in a report prepared by the Association of American Soap & Glycerine Producers (41). This latter report, for some

obscure reason, recommended that phosphate data be expressed as P_2O_5. It became then, the author's habit to express all phosphorus data in this manner.

If one delves into the literature on the subject of phosphorus compounds in aqueous systems, it soon becomes obvious that phosphorus concentrations may be, and are, expressed in a variety of ways. Chief among these methods of expression are: as P_2O_5; as PO_4; and, as P. This lack of uniformity in the presentation of data often leads to significant difficulties in the correlation and interpretation of data from various sources. Perhaps this would be the proper place to suggest that one of the things which would do much to promote understanding, information exchange, and future research in this area would be the adoption of a standard method for the expression of phosphate data. In retrospect, it seems, for a number of reasons, that the proper mode of expressing phosphorus data, at least in studies dealing with biological systems, would be as elemental phosphorus, i.e., as P.

However, the adoption of a standard method for the expression of phosphorus data will probably be a long time in arriving. Therefore, for convenience, the following conversions are given (42):

$$1 \text{ mg/l } P_2O_5 = 0.43 \text{ mg/l P} = 1.34 \text{ mg/l } PO_4$$

a. Soluble Orthophosphate

i. Filter the sample through a 0.45 micron membrane filter.

ii. Place the filtered sample in a 50 ml volumetric flask and dilute to about 40 ml, mix by swirling.

iii. Include a reagent blank with each five samples. Start the determination by adding 3 ml strong acid molybdate solution to the flask containing the reagent blank. Mix by swirling. After 30 seconds, add 4 ml aminonapthol sulfonic acid (ANS) reducing agent, dilute to 50 ml and mix.

iv. At 1 minute, proceed with the next flask, and so on. Read each flask 6 minutes after the introduction of the ANS reducing agent.

v. Read the percent transmission of the samples using a Spectronic "20" colorimeter (Bausch & Lomb, Rochester, New York), at a wavelength of 630 mμ. Use a red filter and red sensitive phototube, and 1/2 inch square cuvettes.

vi. Be sure to include at least on standard with each series of tests.

vii. A calibration curve may be developed, as shown in Figure 3, by reading the percent transmission of a series of standards.

b. Soluble Total Inorganic Phosphate

i. Filter the sample through a 0.45 micron membrane filter.

ii. Place the filtered sample in a 250 ml Erlenmeyer flask, dilute to 40 ml and add 8 ml of 8 N sulfuric acid.

iii. Add 2 boiling chips to each flask, cover with a watch glass, and boil gently for 40 minutes.

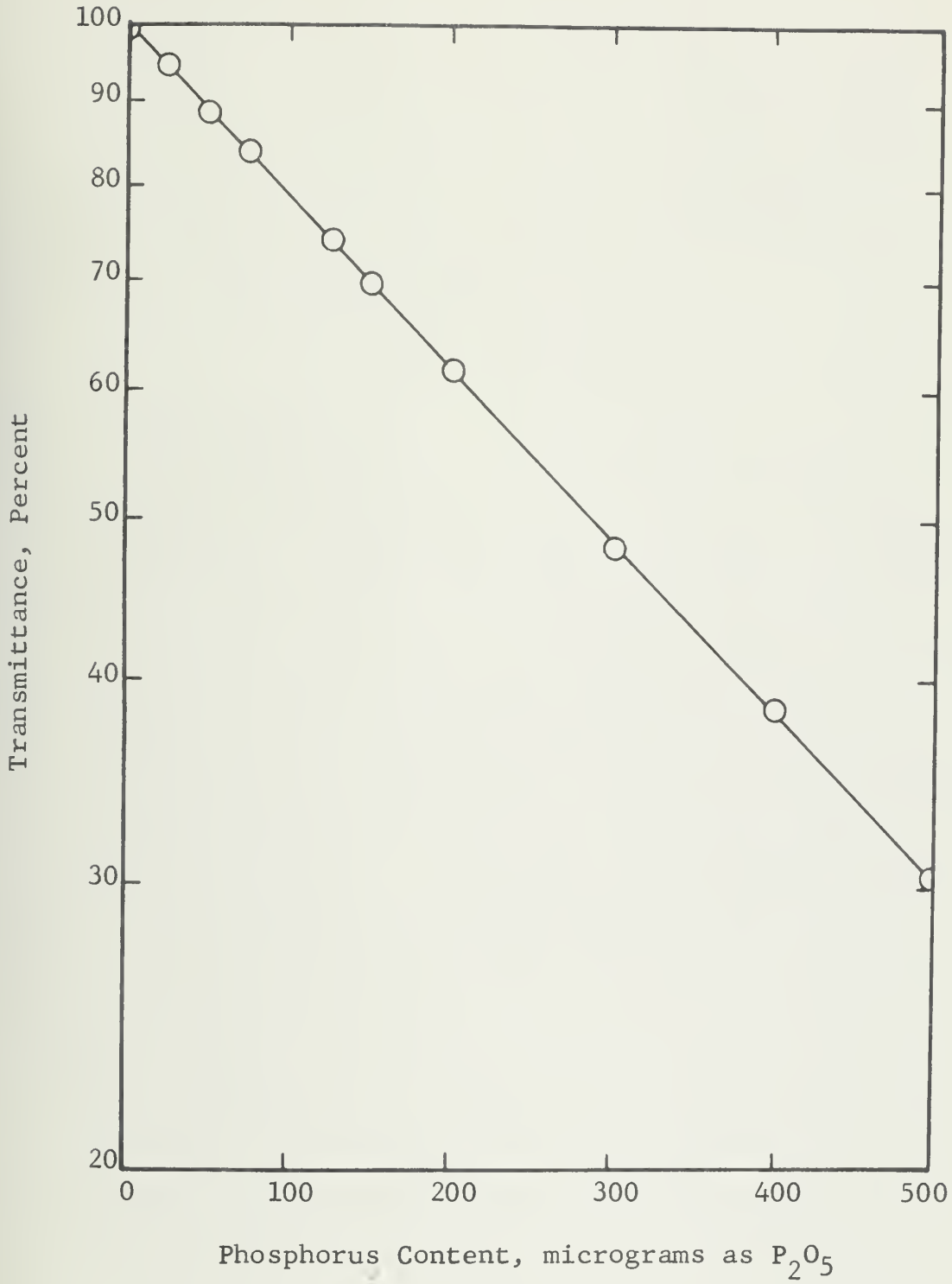

FIGURE 3 CALIBRATION CURVE FOR THE DETERMINATION OF SOLUBLE ORTHOPHOSPHATE.

iv. Allow the samples to cool, neutralize with 8 N sodium hydroxide to the phenolphthalein end point. Add a few drops of 4.5 N sulfuric acid until the color disappears.

v. Transfer the contents of the flasks to 100 ml volumetric flasks, and allow them to cool to room temperature.

vi. Proceed as in steps iii through vi of the soluble orthophosphate determination.

vii. A calibration curve for this method is shown in Figure 4.

c. Total Phosphorus

The total phosphorus content of a sample may include orthophosphate, condensed phosphates, and organic phosphates. The analysis for total phosphorus requires pretreatment of the sample by some type of oxidation and hydrolysis, so as to effect the conversion of the condensed and organic phosphates to the orthophosphate form, which may then be easily measured as outlined above. The pretreatment procedure utilized during the course of this investigation was suggested by Harris and Popat (39), and others (41), and consisted of digesting the sample in the presence of perchloric and nitric acids.

Perchloric acid is particularly suited for the digestion of organic matter because of its high oxygen content and its speed of reaction. It has been noted, however (39), that the addition of a small amount of nitric acid to the perchloric acid-sample mixture will cause the oxidation reaction to begin

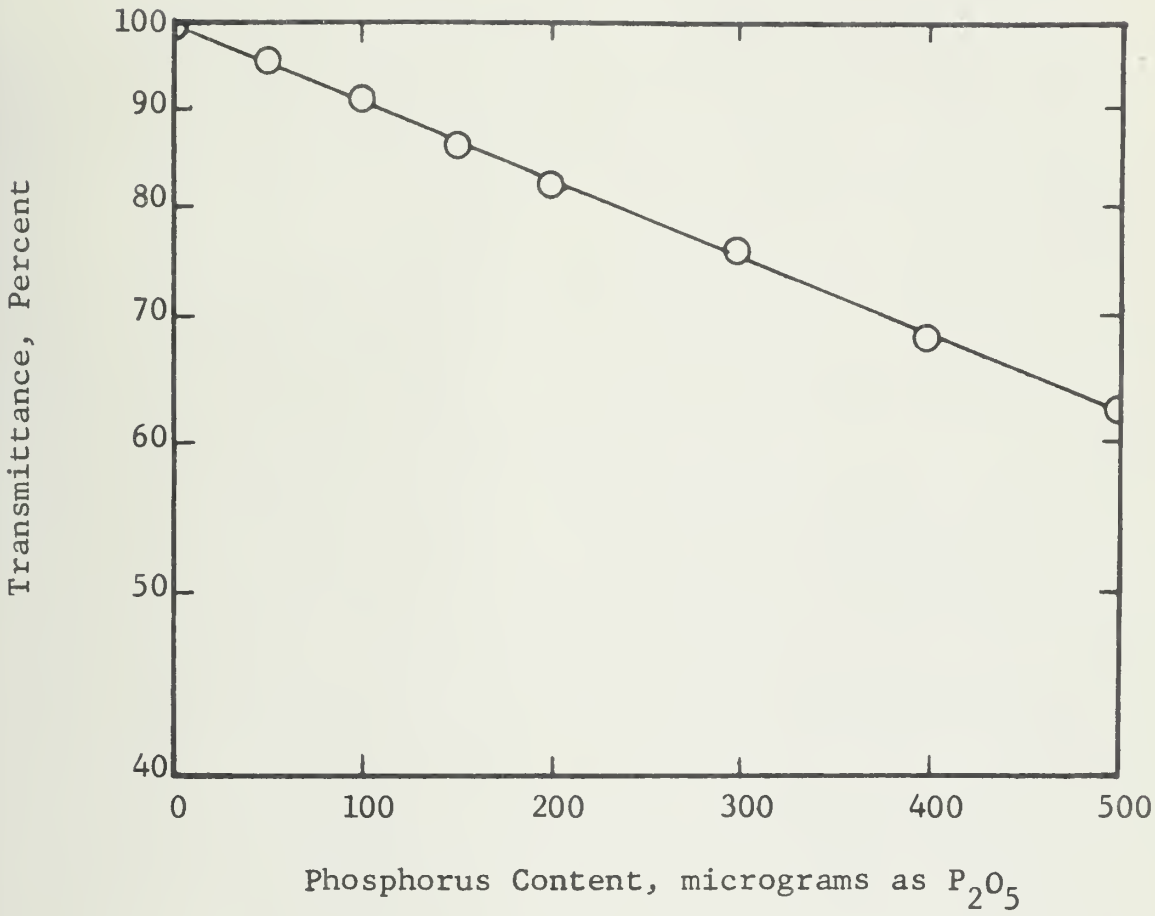

FIGURE 4 CALIBRATION CURVE FOR THE DETERMINATION OF TOTAL INORGANIC PHOSPHATE.

at a lower temperature, and will help to insure a more complete oxidation than is possible with the use of perchloric acid alone.

It has also been reported (39) that the ANS reducing agent, used in the soluble orthophosphate and soluble total inorganic phosphate procedures outlined earlier, does not produce satisfactory results in the presence of perchloric acid. For this reason elon (para-methyl-amino-phenol sulfate), a more suitable reducing agent (39), is used in the total phosphorus procedure outlined below.

i. Place the sample, either filtered or gross, in a 22 x 175 mm Pyrex brand glass test tube.

ii. Add 2 boiling chips, 1 ml of 70-72 percent perchloric acid*, and 2 drops of concentrated nitric acid* to each tube. Mix immediately by gentle swirling.

iii. Heat the samples gently for about 15 minutes, or, until the intense oxidation reaction, characterized by a vigorous foaming action, subsides. Add two more drops of nitric acid to each tube and continue to heat untul the digestion is complete, e.g., dense white fumes of perchloric acid appear in the tube. The resulting solution should be clear; if not, allow to cool slightly, add 1 or 2 ml deionized water and 2 drops of nitric acid and continue to heat until clarity results. Be sure to carry out this operation in a fume hood behind a protective screen!

*See Appendix A.

iv. Allow the clarified samples to cool and transfer the contents of the tubes to 50 ml volumetric flasks. Dilute to about 40 ml.

v. Include a reagent blank and at least one standard with each set of samples. The reagent blank is prepared by adding 1 ml of 70-72 percent perchloric acid to approximately 40 ml of water in a 50 ml volumetric flask. Standards are prepared by adding known amounts of orthophosphate to 50 ml volumetric flasks, and adding 1 ml of 70-72 percent perchloric acid to each flask.

vi. Start the colorimetric analysis by adding 2 ml of 5 percent ammonium molybdate solution* to the flask containing the reagent blank. Mix by swirling. After 30 seconds add 4 ml of elon reducing agent*, dilute to 50 ml and mix.

vii. At 1 minute, proceed with the next flask, and so on. Read each flask 15 minutes after the introduction of the elon reducing agent.

viii. Read the percent transmission of the samples using a Spectronic "20" colorimeter, at a wavelength of 820 mμ. Use a red filter and red sensitive phototube and 1/2 inch square cuvettes.

ix. A calibration curve for this method is shown in Figure 5.

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*See Appendix A.

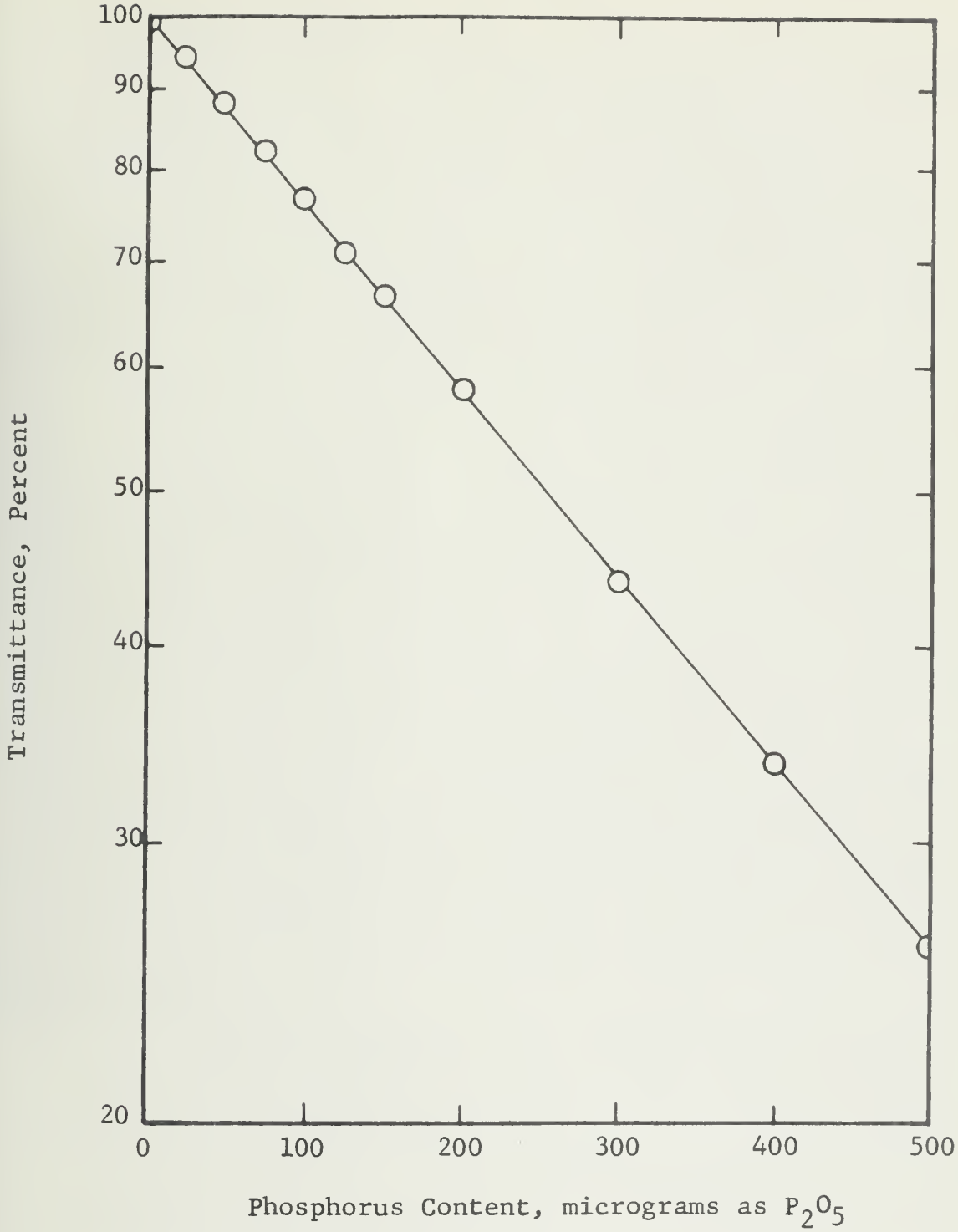

FIGURE 5 CALIBRATION CURVE FOR THE DETERMINATION OF TOTAL PHOSPHORUS.

6. Fractionation Procedure

Experiments were performed during the course of this investigation in which it was desirable to follow the soluble phosphorus taken up by the mixed liquor microorganisms and to identify particular compounds formed by the microorganisms. This was accomplished by observing the distribution of the phosphorus among various broad classes of compounds. It was therefore necessary to carry out a chemical fractionation of the cells.

In such a fractionation, it is desirable to use a procedure which gives "clean" fractions, i.e., a procedure which isolates all of a given class of compounds in one fraction or another. However, if large numbers of samples are to be fractionated, the procedure must also be relatively simple and rapid. The procedure described below is a modification of the procedure outlined by Roberts *et al.* (43) which was developed from the investigations of Schmidt and Thannhauser (44), Friedkin and Lehninger (45), Schneider (46), and Davidson *et al.* (47). This procedure is designed to produce relatively clean fractions with a minimum of manipulation. It has been used extensively in the laboratory of Roberts *et al.* (43), for the fractionation of phosphorus compounds from cells of *Escherichia coli*.

i. Centrifuge a suitable sample of mixed liquor at 17,300 g for 20 minutes at a temperature of 0°C. The centrifuge used during this investigation was a Sorvall RC-2 Refrigerated Centrifuge, manufactured by Ivan Sorvall, Incorporated, Norwalk, Connecticut.

ii. Carefully pour off the supernatant fluid. Rinse the pellet by filling the centrifuge tube with 0.85 percent sodium chloride (NaCl) solution and pouring off immediately without disturbing the pellet.

iii. Wash the cells by resuspending them in 10 ml of 0.85 percent NaCl solution and centrifuging again. Save the supernatant from this operation. This is known as the wash water fraction, and contains only that phosphorus which is associated with the cell but which is on or outside the cell wall.

iv. Resuspend the washed cells in 7 ml of a 5 percent solution of trichloractic acid (TCA) and transfer the suspension to a small 18 x 100 mm glass centrifuge tube. Allow the suspension to stand 30 minutes at 0 to 5°C, then centrifuge and save the supernatant fluid. This supernatant is the cold-TCA-soluble fraction, and contains most of the inorganic cations present within the cell. In addition most of the small phosphorylated molecules, such as ATP, ADP, etc., are recovered in this fraction.

v. Remove any residual fluid remaining from the cold-TCA operation from the walls of the tube with a sterile cotton swab and resuspend the precipitate in 7 ml of a 75 percent ethyl alcohol (ETOH) solution. Keep this suspension at 40-50°C in a water bath or over for 30 minutes and then centrifuge. Save the supernatant fluid. This fraction contains most of the lipids and phospholipids of the cells, and some small amount of protein.

vi. Resuspend the precipitate from the above operation in 7 ml of 5 percent TCA solution. Place the suspension in a boiling water bath for 30 minutes and then centrifuge. A clean glass marble is useful to cover the top of the tube during the boiling, so as to prevent evaporation of the contents. The supernatant fluid from this operation is known as the hot-TCA-soluble fraction. This fraction is composed almost exclusively of nucleic acids, with the possibility of slight amounts of a polysaccharide material.

vii. Save the precipitate remaining from the previous operation. This is termed the residual fraction, and contains the principal protein fraction of the cells.

The total phosphate content of each of these fractions was determined according to the procedure outlined earlier in this report. Investigations revealed that the NaCl, TCA, and ETOH that were present in the various fractions did not interfere with the determination of total phosphate by this method.

V. RESULTS AND DISCUSSION

The experimental results obtained during this investigation are presented below. In order to facilitate the presentation of these data the results of all experiments are presented in graphical or tabular form. A discussion of results is included with the presentation of each phase of the experimental work. Because of the considerable controversy that surrounds the subject of phosphorus uptake by activated sludge systems, all of the experiments reported in this study were repeated, so as to provide confirmation of results. The inclusion of all these data would only serve to make this report more voluminous than it has any right to be, and would not increase its substance to any appreciable degree. Thus, for the most part, only typical results are shown and discussed.

A. Preliminary

Typical data obtained during this investigation are shown in Figure 6. These particular data were developed using sludge from the stock unit, as described in Table 1. These results show a reduction in the concentration of soluble phosphorus of approximately 90 percent during the 24-hour aeration period. More important, however, is the observation that only 50 percent of the total reduction is accomplished during the first 6 hours of aeration. This implies that most existing activated sludge plants, with aeration periods restricted to 6 hours or less, are severely limited in their phosphate removal capability.

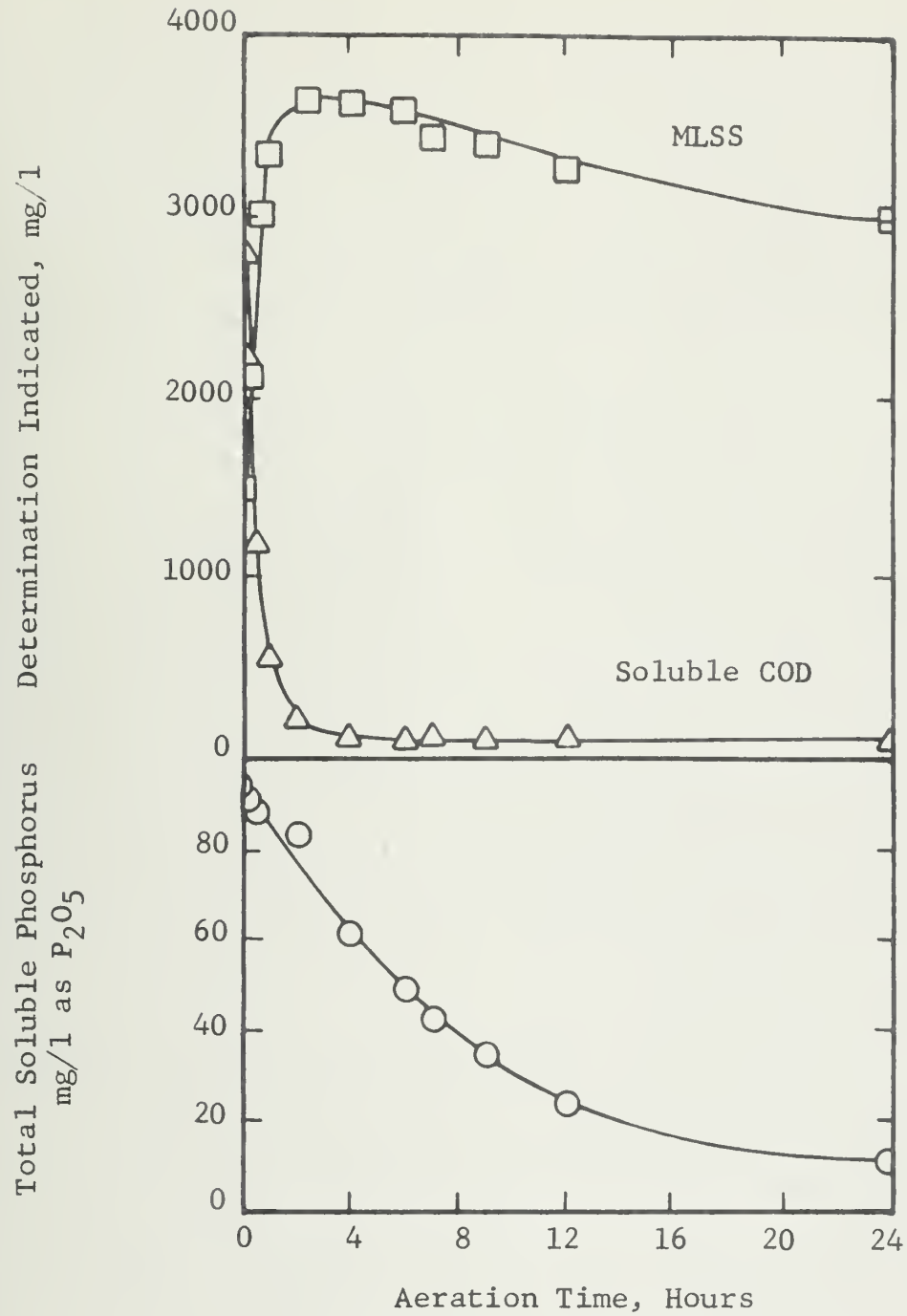

FIGURE 6 VARIATION IN SOLUBLE PHOSPHORUS, SOLUBLE COD AND MLSS DURING AERATION OF ACTIVATED SLUDGE FROM STOCK UNIT.

The data of Figure 6 also show that the uptake of soluble phosphorus is not dependent upon either the rate of soluble COD removal or the rate of change in MLSS concentration. The uptake of soluble COD and the increase in MLSS concentration are essentially complete after 2 hours aeration, whereas the uptake of soluble phosphorus continues throughout most of the aeration period. This observation has implications which will be elucidated later.

One further point needs to be made with regard to the data of Figure 6. Several investigators (12, 22, 24) have reported a "leakage" or secretion of soluble phosphorus from the sludge solids back into solution after 8 to 10 hours aeration. This secretion purportedly resulted in an increase in the soluble phosphorus concentration of the system after prolonged aeration. However, the data of Figure 6 fail to show any evidence of such an occurrence in this system. This observation applies to all of the experiments conducted during this study, as secretion of phosphorus back into solution was never encountered during the course of this investigation.

B. Phosphorus Balance in Activated Sludge

As was previously discussed in Chapter 2, several investigators (12, 14, 22, 24, 25, 27) have reported the uptake of soluble phosphorus by activated sludge. These reports have indicated that with proper operational control the activated sludge process can be utilized to attain significant reductions in the soluble phosphorus content of biologically treatable wastewaters. However, none of these investigations has adequately described the role of the microorganisms present in activated

sludge in the observed disappearance of soluble phosphorus from activated sludge mixed liquor. In other words, what fraction of the observed disappearance of soluble phosphorus from activated sludge mixed liquor can be attributed to the metabolic activity of the microorganisms present in the mixed liquor? The answer to this question would seem to be essential to the complete elucidation of the mechanism of phosphorus removal in activated sludge systems.

Admittedly, Alarcon (22), Srinath et al. (24), and others (12, 14), did consider this question. Their attempts to clarify this point led to their use of mercuric chloride (22), heat (24), and 2,4-dinitrophenol (12, 14) as means of investigating the nature of the phosphate uptake. The results of these investigations indicate that most of this uptake is of a biological nature, i.e., associated with the metabolic activities of the sludge organisms.

There are, however, several means, aside from metabolic uptake, by which soluble phosphorus may be removed from a biological system such as activated sludge mixed liquor. These include adsorption onto the surface of the microorganisms (48, 49, 50), adsorption onto inorganic solids present in the mixture (51), adsorption onto the walls of the container (52), the formation of insoluble precipitates through interaction between phosphate ions and other ions present in the system (17, 53), as well as the adsorption of phosphate ions onto air bubbles and their subsequent removal by entrapment in either the foam generated on the surface of the system, or in aerosols leaving the system (54). In addition, these previous investigations (12, 14, 22, 24) were, for the most part, concerned only with the uptake of soluble orthophosphate. However, it is reasonable to assume

that the orthophosphate originally present in the system may be converted, through microbial action, into other forms of soluble phosphorus. In such a case, because of the nature of the orthophosphate determination, soluble orthophosphate will appear to have been removed, even though there is actually no net change in the total soluble phosphorus content of the system. In like manner, the possible conversion of other forms of soluble phosphorus to the ortho form must also be considered when performing a soluble phosphorus balance on such a system. None of the investigations cited previously (12, 14, 22, 24, 25, 27) reported any consideration of these possibilities.

There are, in short, several mechanisms which must be considered and evaluated in order to determine the relationship between the disappearance of soluble phosphorus from a microbial system and the metabolic activity of the system. The methods, i.e., mercuric chloride, heat, and 2,4-dinitrophenol, used by other investigators (12, 14, 22, 24) to evaluate this relationship are subject to criticism because although they are known to produce an effect on the metabolic activity of the cells, they may also produce an effect on other possible mechanisms by which soluble phosphorus may be removed from the system. It therefore seemed necessary to consider, by some other means, the role that microorganisms play in the observed disappearance of soluble phosphorus from activated sludge mixed liquor. To this end a series of experiments were performed with a view toward the systematic evaluation of the efficacy of these proposed mechanisms in the removal of soluble phosphorus from microbial systems.

1. Evaluation of Suggested Mechanisms of Soluble Phosphorus Uptake by Activated Sludge

Four different experimental systems were employed for this phase of the study. These systems were designed so as to permit an evaluation of the influence of the various components of activated sludge mixed liquor, i.e., biological solids, organic substrate, and inorganic nutrients, on the soluble phosphorus removal capacity of the total system.

One of these systems was an experimental laboratory scale activated sludge unit of the fill and draw type, composed of biological solids, organic and inorganic nutrients, and water, as described earlier in Table 1. The other three systems were modifications of this total system in that they were "blanks," i.e., void of one or more of the ingredients considered to be essential to the proper functioning of the activated sludge process. These "blanks" were prepared as follows. One of the blanks consisted of all the above mentioned components except biological solids. A second blank was devoid of both biological solids and organic substrates, and thus contained only inorganic nutrients and water. The third blank contained only soluble phosphorus, buffering material, and water. All systems were operated in an identical manner with regard to pH, temperature, and air flow rate. A description of these systems is given in Table 2.

Using these experimental systems it was possible to evaluate the effect of the biological solids, i.e., microorganisms, on the disappearance of soluble phosphorus from activated sludge mixed liquor, without the use of materials that are foreign to the system, such as poisons or

TABLE 2

DESCRIPTION OF EXPERIMENTAL UNITS USED FOR PHOSPHORUS BALANCE IN ACTIVATED SLUDGE UNITS

Ingredients	Initial Concentration (mg/l except as indicated) Activated Sludge System	Blank No. 1	Blank No. 2	Blank No. 3
Biological solids	1500	0	0	0
Glucose, as COD	2400	2400	0	0
Yeast extract, as COD	600	600	0	0
NH_4Cl	600	600	600	0
$MgSO_4 \cdot 7\ H_2O$	250	250	250	0
$FeSO_4 \cdot 7\ H_2O$	10	10	10	0
$MnSO_4 \cdot H_2O$	10	10	10	0
$CaCl_2 \cdot 2\ H_2O$	10	10	10	0
KH_2PO_4	68	68	68	68
$Na_2HPO_4 \cdot 7\ H_2O$	134	134	134	134
Na_2CO_3	2650	2650	2650	2650
Tap water	100	100	100	0
Distilled water, to make one liter of solution	yes	yes	yes	yes
pH	7.5	7.5	7.5	7.5
Waste schedule percent of mixed liquor per day	50	-	-	-
Initial loading rate (F:M)	2	-	-	-
Air flow rate $ft^3/(hr)(l)$	4	4	4	4

inhibitors. This approach also permitted an evaluation of the extent to which several other proposed phosphorus uptake mechanisms contribute to the total soluble phosphorus uptake of activated sludge systems.

Typical data obtained during this phase of the investigation are shown in Figure 7. These particular data were developed using the experimental activated sludge unit as described in Table 2.

These results show a reduction in the concentration of soluble orthophosphate of approximately 90 percent during the 24-hour aeration period. These data also show that the total soluble phosphorus concentration is reduced by approximately the same amount, i.e., 90 percent, during the same period of aeration. Inspection of these data reveals that the rate of uptake of total soluble phosphorus is almost identical to the rate of soluble orthophosphate uptake, except during the first hour of aeration. The difference in these two uptake rates during the early stages of aeration is attributed to the rapid conversion of condensed and/or organic phosphates, originally present in the yeast extract feed solution, to the ortho form. Such a conversion is known to occur in aqueous solutions, and the rate of conversion is reportedly influenced by bacterial action, being higher in sewage than in pure water (55). The data of Figure 7 indicate that this conversion is essentially complete in this system by the end of the first hour of aeration, and that thereafter the rate of uptake of total soluble phosphorus is identical to the rate of soluble orthophosphate uptake. From these data it may be concluded that the disappearance of total soluble phosphorus from solution is caused by the uptake of soluble orthophosphate.

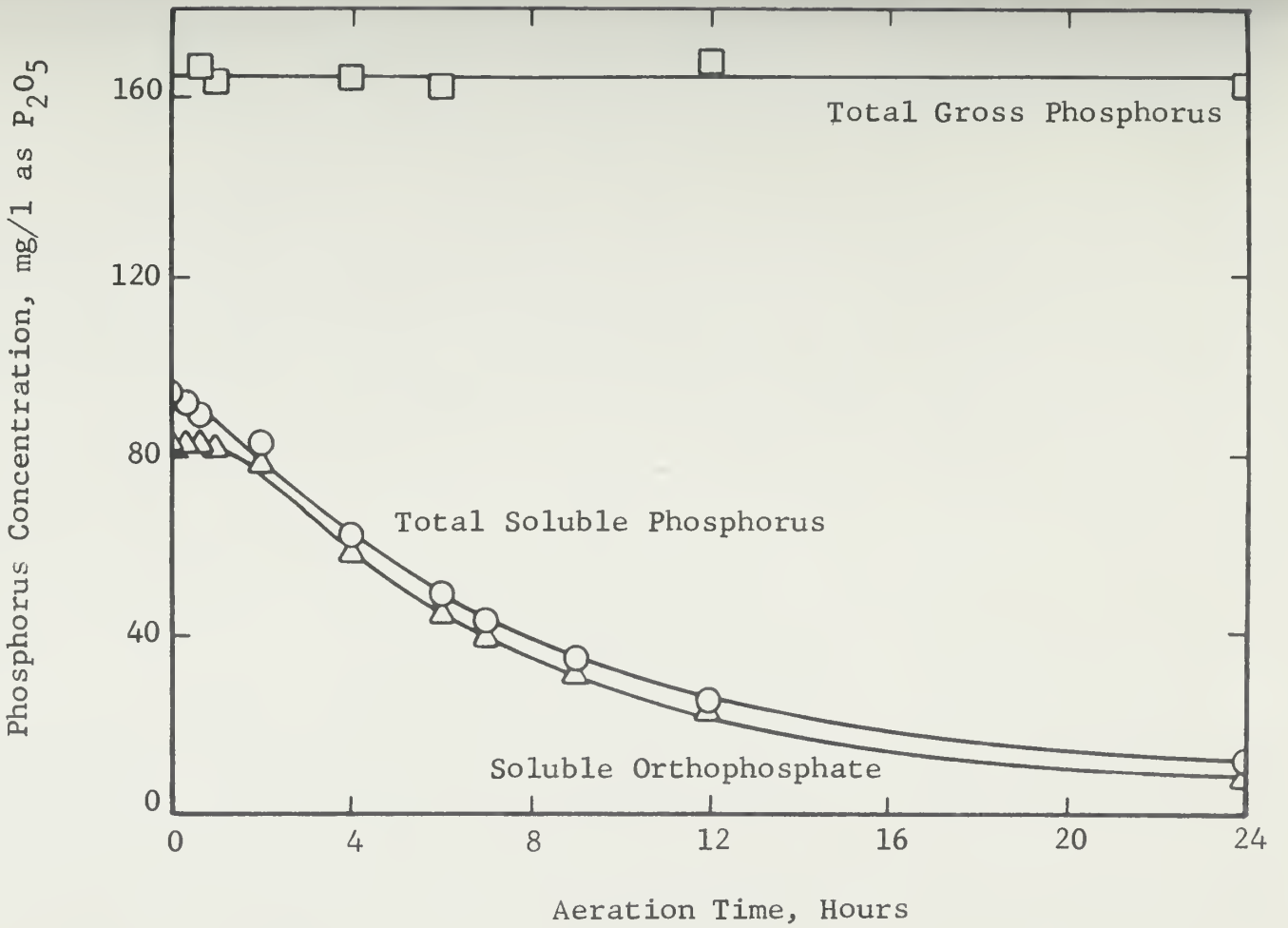

FIGURE 7 TOTAL SOLUBLE PHOSPHORUS, SOLUBLE ORTHOPHOSPHATE, AND TOTAL GROSS PHOSPHORUS DURING AERATION OF ACTIVATED SLUDGE FROM STOCK UNIT.

This last observation has dual significance. First, the relationship between soluble orthophosphate uptake and the uptake of total soluble phosphorus indicates that the disappearance of soluble orthophosphate from activated sludge systems, observed by other investigators (12, 14, 22, 24), was in fact a true removal of soluble phosphorus from the system, and not merely a conversion of soluble orthophosphate to some other form of soluble phosphorus. This finding obviates one of the objections raised earlier in this discussion, and rules out one of the possible mechanisms of soluble orthophosphate removal in activated sludge, namely the conversion of soluble orthophosphate to other forms of soluble phosphorus.

Second, it has been reported that the only form of phosphorus that is unequivocably known to be utilized by algae is orthophosphate (56). The finding that the uptake of soluble phosphorus by activated sludge is actually a result of the uptake of soluble orthophosphate suggests that this uptake is of immediate and positive benefit in the control of algae blooms in receiving waters.

The data of Figure 7 also yield information on another point of importance. It was suggested earlier that soluble phosphorus might be removed from activated sludge mixed liquor by the sorption of phosphate ions onto the surface of air bubbles. In this way, soluble phosphorus might be stripped from the body of the system by incorporation into foams or aerosols. A similar phenomenon was observed in the laboratory by Baylor et al. (54) while aerating sea water. A mechanism of this type might account for a portion of the uptake of soluble phosphorus by activated sludge that has been observed by other investigators (12, 14, 22, 24).

Figure 7 also presents data on the total gross phosphorus (TGP) concentration, i.e., the total phosphorus concentration of unfiltered mixed liquor, of the system used in this study. If the sorption mechanism suggested above contributed appreciably to the reduction in soluble phosphorus observed in this system, the TGP concentration would of necessity decline throughout the aeration period. However, the data clearly show that there was no measurable reduction in the TGP concentration during aeration. Thus this mechanism does not appear to be of significance to the observed disappearance of soluble phosphorus from this system.

A comparison of the soluble phosphorus uptake in the activated sludge unit, to that in the various "blank" units used in this phase of the study is shown in Figure 8. These data demonstrate the importance of biological solids, i.e., microorganisms, in this uptake. These data show no measurable change in the soluble phosphorus concentration of any of the "blank" systems throughout the duration of the experiment. The only system which was able to effect a reduction in soluble phosphorus was the unit containing biological solids. These results, coupled with the data of Alarcon (22), Srinath et al. (24), and others (12, 14), clearly indicate that the mechanism of soluble phosphorus uptake by activated sludge is dependent upon the presence of microbial solids for its operation.

Inspection of the data shown in Figure 8 also shows that other mechanisms which have been proposed to explain this phenomenon, e.g., sorption onto the walls of the container, sorption onto inorganic solids present in the system, and the formation of insoluble precipitates of phosphorus, do not play a significant role in the uptake of soluble phosphorus by the activated sludge systems used in this investigation. However,

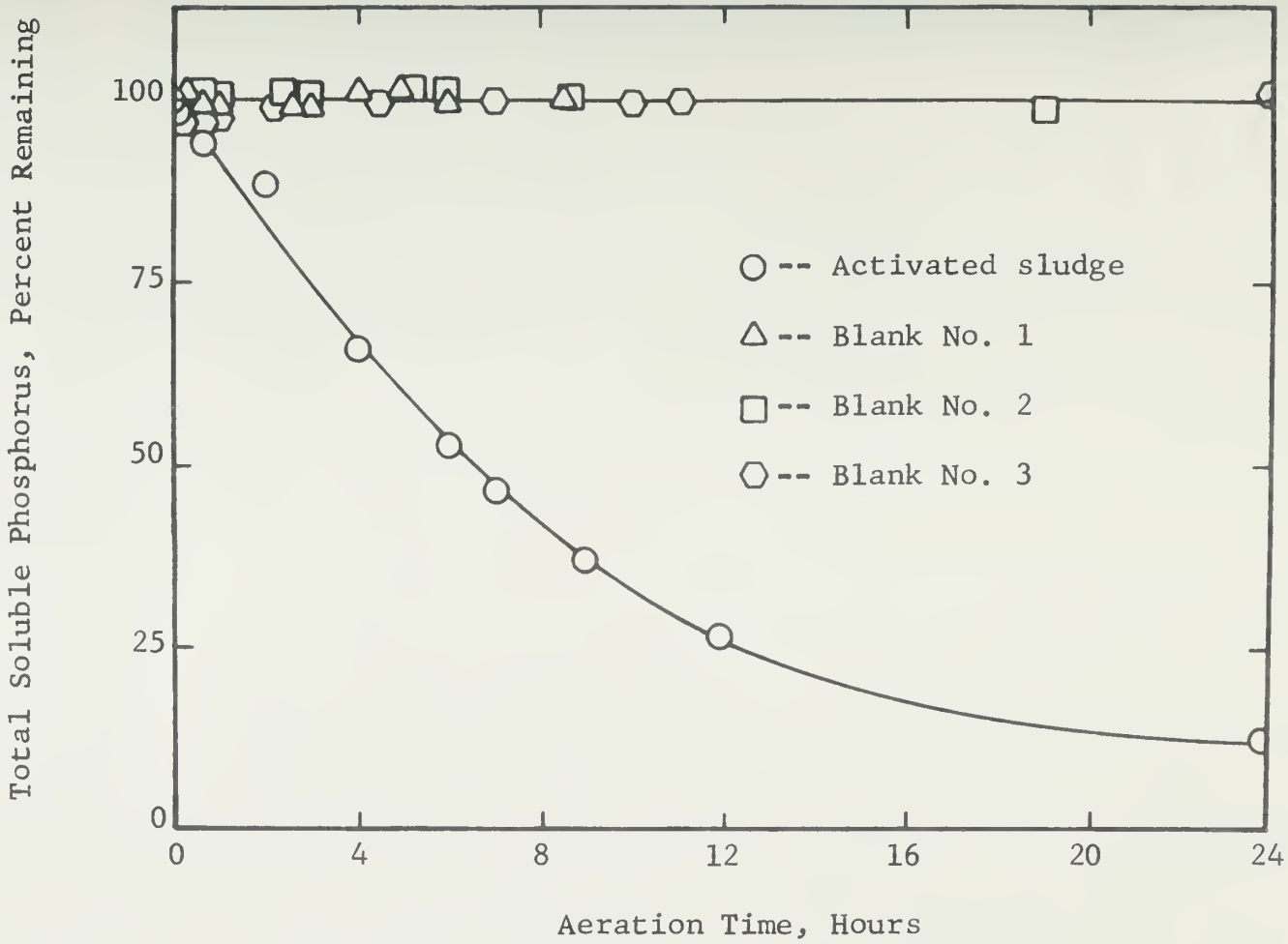

FIGURE 8 SOLUBLE PHOSPHORUS UPTAKE IN ACTIVATED SLUDGE AND BLANK SYSTEMS DURING AERATION.

it should be pointed out that the activated sludge units used in this study were maintained on a synthetic, soluble substrate. Thus, these systems received only small, carefully limited amounts of the cations which are known to participate in the precipitation of phosphorus from aqueous solutions, i.e., iron, calcium, magnesium, manganese, and aluminum. In addition, these systems were essentially devoid of inorganic suspended solids, such as clays and silts. In the more general case of an activated sludge plant treating a domestic or industrial waste, it is reasonable to assume that at least two of the aforementioned mechanisms of phosphorus removal, i.e., sorption onto inroganic solids, and the formation of insoluble precipitates of phosphorus, might indeed play a significant role in the overall removal of soluble phosphorus from the waste. The magnitude of this contribution would of course depend upon many factors, such as the concentration and characteristics of the suspended inorganic solids, and the concentration of the various metallic ions present in the waste, as well as the pH of the system, and the solubilities of the metalphosphate precipitates, to name a few. All that is being claimed here is that, probably because of the characteristics of the synthetic medium, these mechanisms are not significant in the removal of soluble phosphorus observed in the activated sludge units used in this study.

2. Cellular Phosphorus Balance

The evidence presented thus far in this report has demonstrated the predominant role that microorganisms play in the removal of soluble phosphorus from activated sludge mixed liquor. However, a question persists as to the type of microbial activity responsible for this removal.

Does this uptake result from the storage of phosphorus on or within the cells, or is it the result of the metabolic assimilation of phosphorus into new cellular material? In other words, is it a "luxury" uptake and storage, or is it actually controlled by the metabolic needs of the cells?

In order to answer this question it was necessary to trace the soluble phosphorus taken up by the mixed liquor microorganisms, and to observe its distribution among certain broad classes of phosphorylated compounds formed by the microorganisms. In this way it was possible to determine any change in the phosphorus content associated with these compounds that occurred during the uptake of soluble phosphorus. The fractionation procedure used during this phase of the investigation was described in Chapter 4. These experiments were performed using sludge from the stock unit, as described in Table 1.

Typical results obtained during this phase of study are presented in Table 3, which shows the fractions studied and the phosphorus concentration of each fraction after various periods of aeration. These data show a predictable decrease in the system's soluble phosphorus concentration throughout the aeration period, along with a corresponding increase in the phosphorus concentration of the various cellular fractions. Inspection of these data reveals that the phosphorus concentration of each cellular fraction was approximately doubled during the aeration period. Although not shown here, a doubling of the system's MLSS concentration was also recorded during this period. These two facts indicate that the uptake of soluble phosphorus in this system is associated with the synthesis of new cellular material, i.e., associated with a metabolic uptake.

TABLE 3

CELL FRACTIONATION STUDIES, PHOSPHORUS BALANCE IN ACTIVATED SLUDGE

Time (min)	Phosphorus Concentration (mg/l as P_2O_5)						
	Total Gross	Total Soluble	Total Insoluble				
			0.85% NaCl Wash	Metabolic Intermediates (Cold TCA)	Phospholipids (Hot ETOH)	Nucleic Acids (Hot TCA)	Protein (Residue)
3	186	87	0.6	19.8	1.5	56.7	3.9
30	174	81	3.1	26.5	4.9	54.1	4.5
60	146	77	0.8	18.5	4.5	47.0	3.5
126	163	60	1.9	26.9	3.5	73.6	4.1
240	187	40	1.5	24.5	2.9	86.5	9.1
360	186	22	0.9	30.5	3.0	107.8	8.4
615	168	6	0.5	26.3	8.1	109.0	7.5
720	180	3	1.0	41.3	8.6	104.2	8.1
1440	177	2	0.8	40.0	4.1	117.2	9.9

Figure 9 is a graphical presentation of some of the data given previously in Table 3. These results show the soluble phosphorus uptake plotted along with the total insoluble phosphorus, i.e., the sum of the phosphorus content of all cellular fractions, including the wash solution. It is apparent from this figure that the reduction in the soluble phosphorus content of the system, i.e., soluble phosphorus uptake, is closely paralleled by an increase in the system's cellularly associated phosphorus. Indeed, it can be seen that essentially all of the soluble phosphorus removed from the system is recoverable in the system's various cellular fractions. This is strong evidence of the association between the cells' biosynthetic activity, i.e., synthesis of cellular material, and the uptake of soluble phosphorus.

The phosphorus content of the nucleic acid fraction is also shown in Figure 9, and demonstrates that the uptake of soluble phosphorus in these systems results in a corresponding increase in the phosphorus content of the cells' nucleic acid fraction. Again, this is a strong indication that the soluble phosphorus uptake observed in these systems is the result of the cells' biosynthetic activity.

For convenience, selected data from Table 3 are presented in Table 4, showing the distribution of insoluble phosphorus in the solution used for washing the cells, as well as in the various cellular fractions. This distribution was computed as percent of the total insoluble phosphorus, i.e., total gross phosphorus minus total soluble phosphorus, and was based on the average TGP of the system.

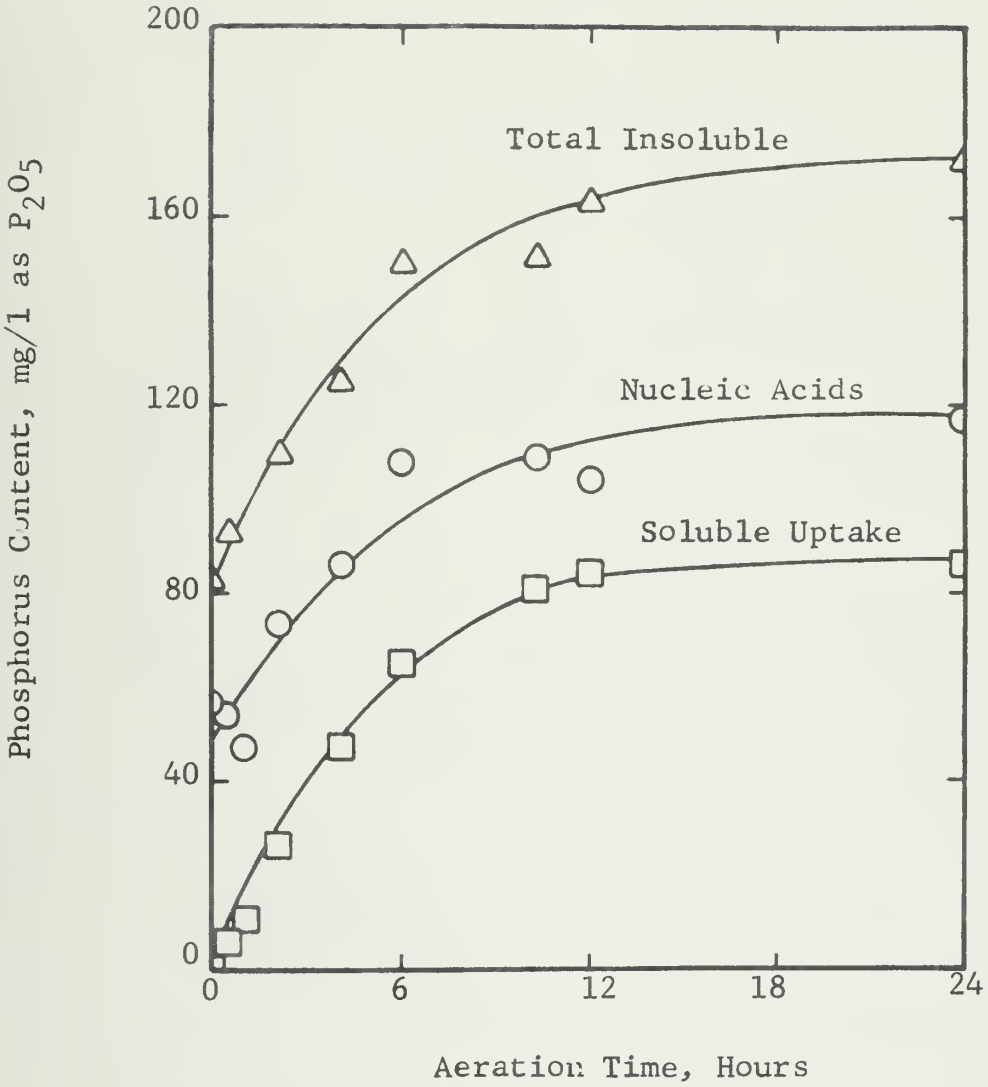

FIGURE 9 SOLUBLE PHOSPHORUS UPTAKE, CELL FRACTIONATION STUDIES.

TABLE 4

CELL FRACTIONATION STUDIES, DISTRIBUTION OF INSOLUBLE PHOSPHORUS

Time (min)	Phosphorus Content (Percent of Total Insoluble Phosphorus*)					
	0.85% NaCl Wash	Metabolic Intermediates (Cold TCA)	Phospholipids (Hot ETOH)	Nucleic Acid (Hot TCA)	Protein (Residual)	Percent Recovery
3	0.7	22.8	1.7	65.2	4.5	94.9
30	3.2	28.5	5.2	58.2	4.8	99.9
60	0.8	18.9	4.6	48.0	3.6	75.9
126	1.7	23.6	3.0	64.6	3.6	96.5
240	1.1	18.4	2.2	64.5	6.8	93.0
360	0.6	20.1	2.0	71.0	5.5	99.2
615	0.4	15.7	4.8	64.6	4.5	90.0
720	0.6	24.1	5.0	61.0	4.7	95.4
1440	0.5	23.2	2.4	68.1	5.7	99.9
mean	1.1	21.7	3.4	62.8	4.9	93.9

*based on average total gross phosphorus

These data show that essentially all of the phosphorus removed from solution during aeration can be traced to the cells composing the activated sludge. Table 4 shows that an average of 93.9 percent of the total soluble phosphorus removed from the system was recovered in the various cellular fractions.

Only small amounts of the soluble phosphorus taken up by the cells could be removed by washing with isotonic saline solution. There is some doubt, however, whether or not washing with saline solution will effectively remove phosphorus that might be adhering to the surface of the cells. For this reason it should be pointed out that additional research (57) in this area has been conducted in the Sanitary Engineering Laboratories of the University of Maine, to determine if phosphorus can be removed from the cells by washing with slightly acid solutions. These studies have shown that washing with solutions having pH values as low as 5.0, for 15 minutes, does not produce a significant reduction in the amount of phosphorus associated with the cells. It was found that pH values on the order of 2 to 3 were necessary to achieve significant release of phosphorus from the cells. All of this information seems to indicate either that only very small quantities of phosphorus are held on the cell surfaces, or that the bonds holding the phosphorus molecules to the cell surfaces are quite strong.

Table 4 also shows that most of the phosphorus removed from solution was distributed in various fractions within the cell, principally in the form of nucleic acids and low molecular weight intermediates. These two fractions contain approximately 60 percent and 20 percent, respectively, of the cells' total phosphorus.

These data also show that there was no significant change in the distribution of phosphorus within the cells during the aeration period. Apparently the phosphorus that was removed from solution was used for the synthesis of new cellular material having approximately the same phosphorus distribution as the older cells. In other words, there appeared to be a smooth transfer of phosphorus from the solution into the various cell fractions, with no discernable increase in any fraction's contribution to the total insoluble phosphorus content of the system. Thus it may be concluded that the uptake of soluble phosphorus by these systems is not the result of an accumulation or storage of phosphorus within a particular cellular fraction but rather results from the cells' use of phosphorus in the synthesis of new cellular materials.

C. Influence of Mixed Liquor Suspended Solids Concentration

Several investigators have referred to the role of MLSS in the uptake of soluble phosphorus by activated sludge systems. However, for several reasons, the influence of MLSS concentration on this uptake has remained in question.

Srinath et al. (24) were apparently the first to investigate the effect of the solids content of the mixed liquor on phosphorus uptake. As mentioned earlier, they reported that maximum uptake was produced by a mixed liquor containing 20 to 30 percent return sludge on a volume basis. This liquor was reported to have effected a 90 percent reduction in the soluble phosphorus concentration of the settled supernatant after six hours aeration. On the other hand, aerated raw sewage,

containing no return sludge, took up practically no soluble phosphorus during the same period. Unfortunately, no data were presented as to the MLSS concentration of these sludges.

In 1962, using return activated sludge and feeding effluent from a primary settling basin, Feng (25) evaluated the effect of five different MLSS concentrations and two different aeration rates on the uptake of soluble orthophosphate by activated sludge. With the higher rate of aeration there was, in general, greater orthophosphate removal in systems having a low MLSS concentration. There are, however, certain inconsistencies in Feng's data. Moreover, at the lower aeration rate, the different MLSS concentrations had no discernable effect on orthophosphate reduction.

Sekikawa et al. (27) using an aeration rate almost twice as great as Feng's (25) highest rate, found the concentration of MLSS to have little effect on orthophosphate removal.

Levin (14), and Levin and Shapiro (12) found that a small amount of return sludge in raw sewage dramatically improved orthophosphate uptake over that obtainable in raw sewage alone. However, they reported a lack of proportionality between the percent of return sludge used in their systems and the observed orthophosphate uptake of the system. Data concerning the actual concentration of MLSS in these sludges were not included in their reports.

It should also be pointed out that, insofar as can be determined, none of these investigators (12, 14, 24, 25, 27) used acclimated systems in their studies. There seemed to be then, adequate reasons for investigating the effect of MLSS concentration on the uptake of soluble phosphorus by activated sludge.

Five different activated sludge units were initiated for this study. The feeding and maintenance of these units were exactly as described earlier for the stock unit. Substrate and nutrient concentrations, and other operating parameters, were identical with those described in Table 1, except for the wasting schedule. By wasting different amounts of mixed liquor from each unit, different MLSS concentrations could be maintained in the various units, even though they received the same feed. Table 5 shows the wasting schedule, loading rate and other pertinent data for each of these units. As soon as these units had become acclimated to the desired conditions, data collection was begun.

The data on COD removal obtained from these units are summarized in Figure 10. From these data it can be seen that the initial rate of COD uptake varied from unit to unit, increasing as the F:M values became smaller. However, in each unit, the soluble COD remaining eventually reached an equilibrium value of approximately 100 mg/l. Thus, each unit was successful in taking up approximately 2900 mg/l COD during the aeration period.

Figure 11 presents the data on the increase in MLSS concentration obtained from the units used in this portion of the study. These data show a situation similar to that observed in the data on COD uptake, i.e., the initial rate of MLSS increase became larger as the F:M values were decreased. However, it can also be seen that, as aeration was continued, the MLSS concentration of each unit eventually reached an equilibrium value, which was, in each case, approximately 1500 mg/l higher than the initial MLSS value. This net increase in MLSS concentration is representative of the net metabolic synethsis of the system. Thus, in each

TABLE 5

OPERATING PARAMETERS: MLSS CONCENTRATION STUDIES

Unit Number	1	2	3	4	5
Wasting Schedule (% of mixed liquor/day)	16	20	33	50	67
Initial F:M	0.38	0.50	1.00	2.00	4.00
Initial COD (mg/l)	3000	3000	3000	3000	3000
Initial MLSS (mg/l)	8000	6000	3000	1500	750

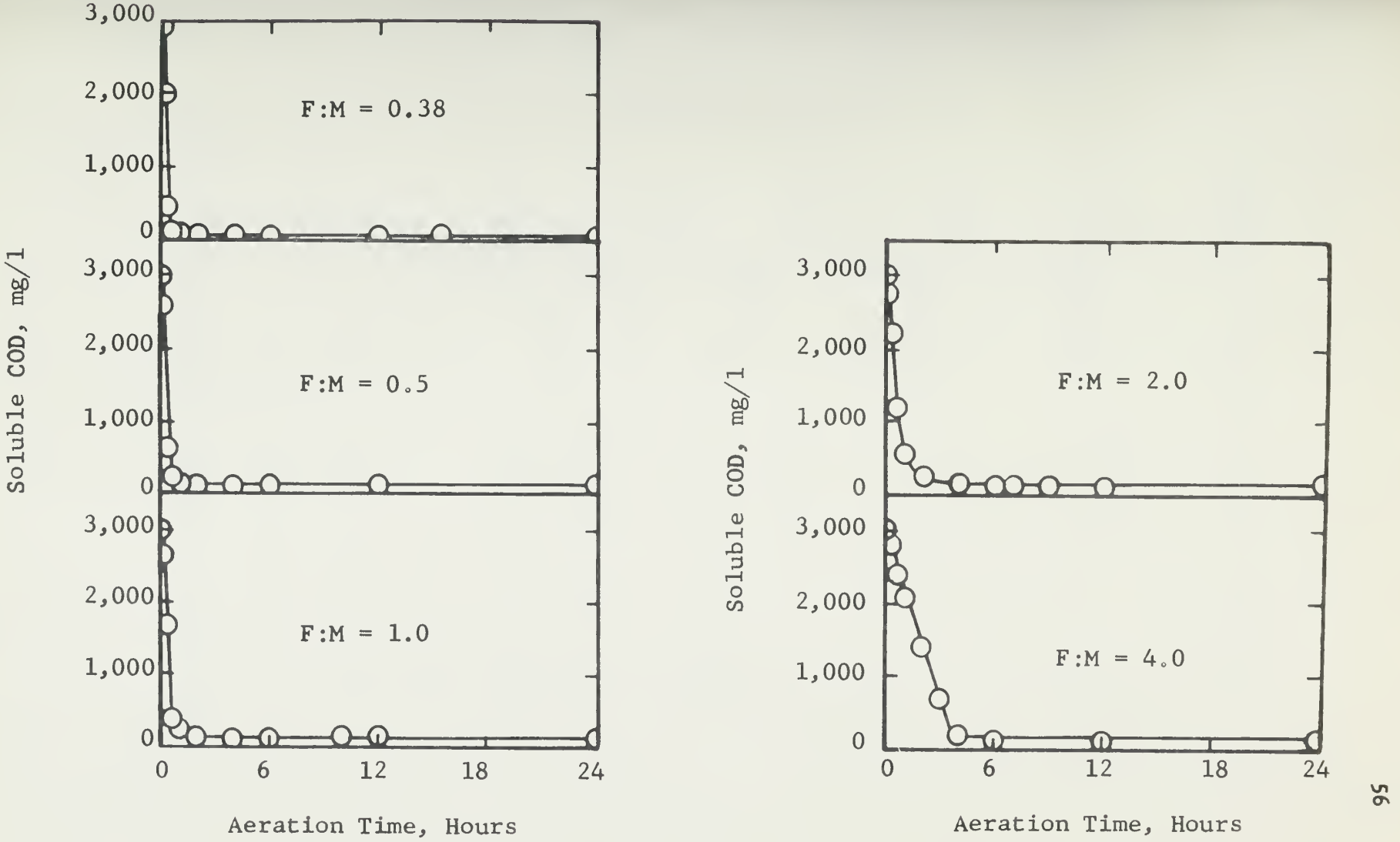

FIGURE 10 VARIATION IN SOLUBLE COD DURING AERATION OF ACTIVATED SLUDGE: MLSS STUDIES.

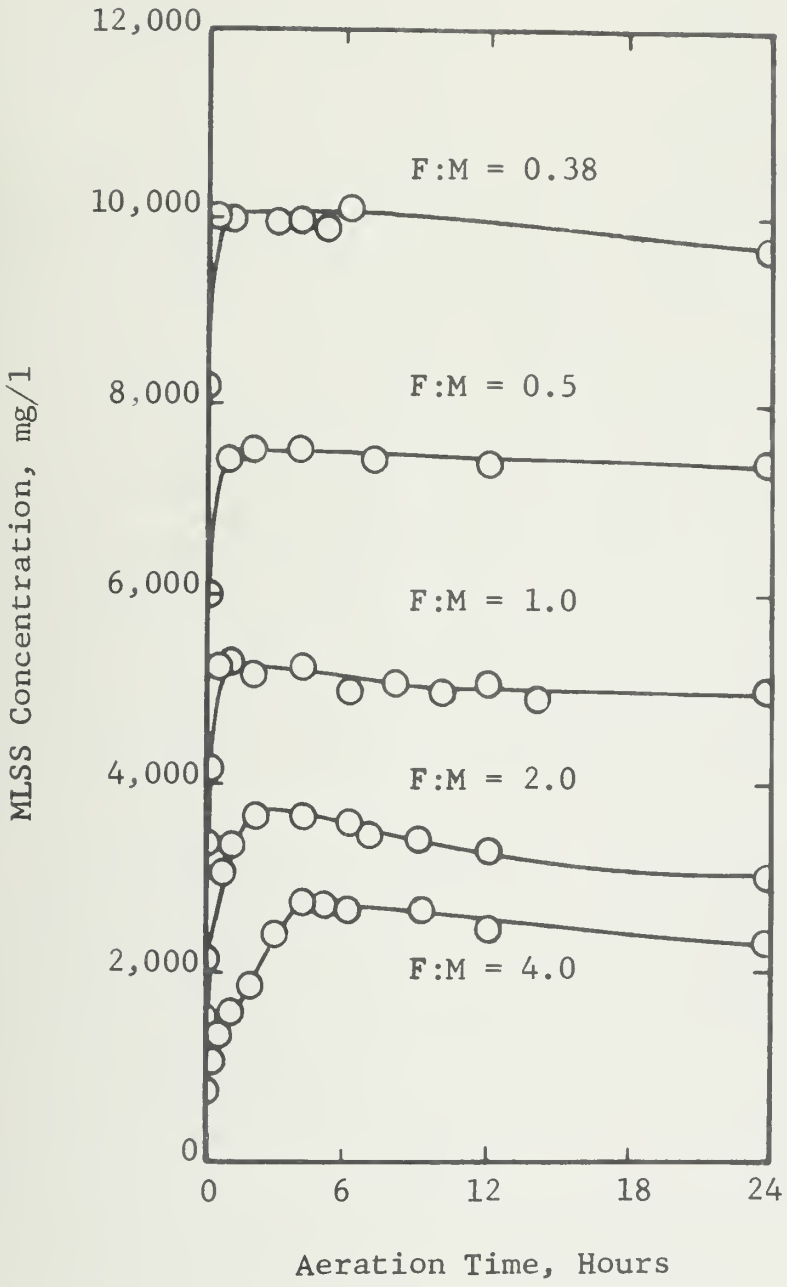

FIGURE 11 VARIATION IN MLSS DURING AERATION OF ACTIVATED SLUDGE: MLSS STUDIES.

system, approximately 2900 mg/l of COD were taken up, and approximately 1500 mg/l of MLSS were synthesized, during the aeration period. In other words, each unit was able to use about 50 percent of its available substrate for the synthesis of new cellular material, over the 24 hour aeration period. This value compared favorably with synthesis values given by other sources (58, 59). Moreover, this value is supported by data on initial COD, and initial and final MLSS concentration, collected on these units over a period of several months.

Figure 12 presents a summary of the data on phosphorus uptake obtained from these units. It may be seen from this plot that there was no discernable difference in either the rate or extent of soluble phosphorus uptake by these different systems. These data indicate that a change in the concentration of MLSS in the aeration basin of an actual treatment plant would have little effect on that plant's ability to take up soluble phosphorus.

The data presented in Figures 10, 11, and 12, also show that the rate of soluble phosphorus uptake in these systems, being essentially constant in each unit, is not related to either the rate of soluble COD uptake, or the rate of increase in MLSS concentration. However, when the rate of uptake or increase in these parameters is ignored, and comparisons are made on the basis of total uptake or increase during the 24 hour aeration period, it becomes apparent that these new parameters, i.e., total soluble phosphorus uptake, total soluble COD uptake, and net increase in MLSS increase, are somehow related.

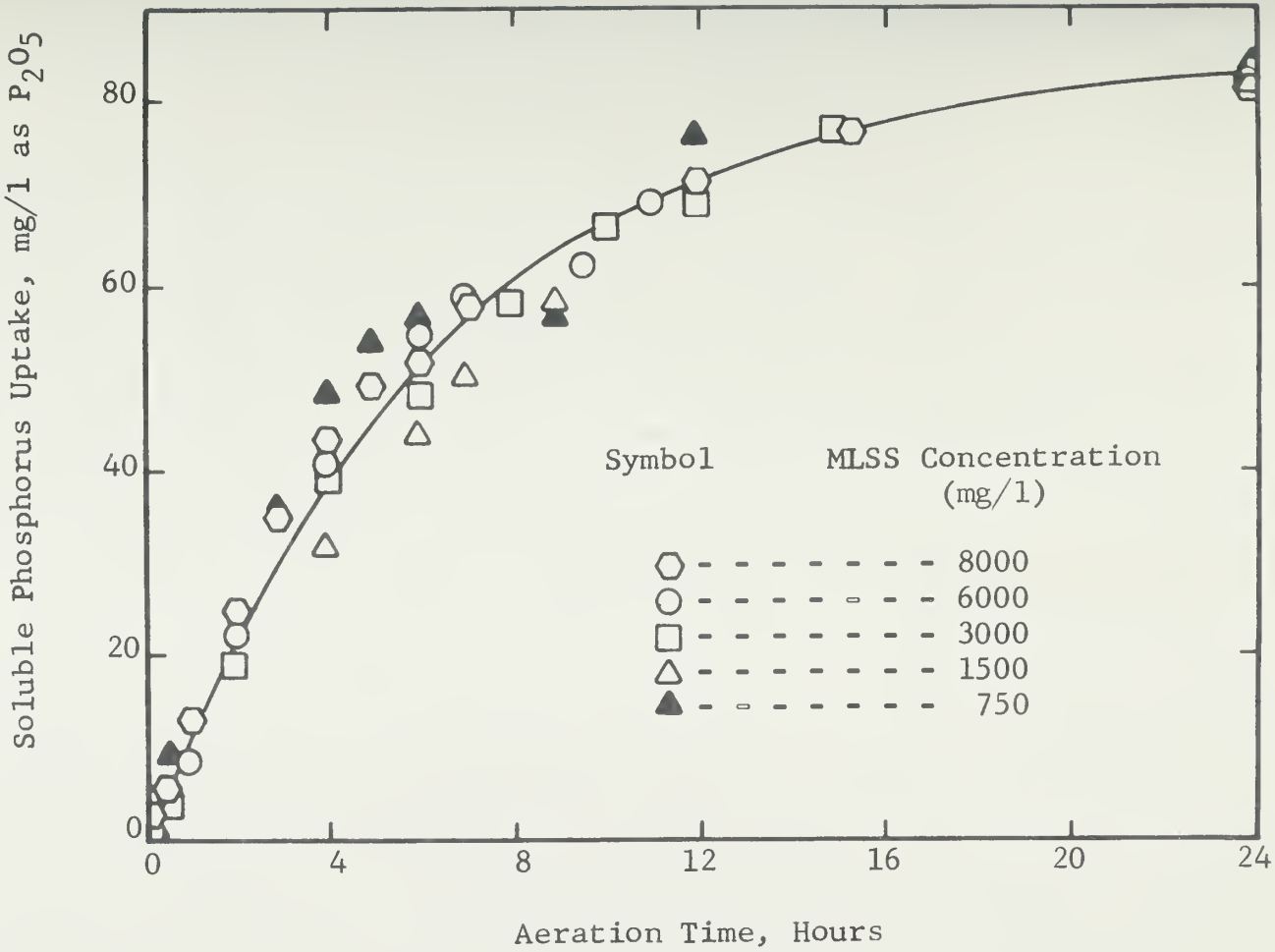

FIGURE 12 SOLUBLE PHOSPHORUS UPTAKE AS A FUNCTION OF MLSS CONCENTRATION.

For example, Figure 12 shows that the uptake of soluble phosphorus in all five units was essentially completed by the end of the 24 hour aeration period. Furthermore, each of these units took up essentially the same total amount of soluble phosphorus during this period. Table 6 is a further summarization of the data presented in Figures 10, 11, and 12, showing the total amount of soluble phosphorus, and soluble COD taken up by each unit, and the net increase in MLSS concentration in each unit during the 24 hour aeration period. It may be noted from these data that the ratio of net MLSS increase to total phosphorus uptake (MLSS increase:P uptake) was essentially the same for each unit, the average value being approximately 42:1. This fact will be elaborated upon later. For now it is sufficient to say that, in all five units, regardless of F:M ratio, approximately 1 unit of soluble phosphorus was taken up for each 42 units of MLSS that was synthesized during the 24 hour aeration period.

There is one further feature of these data which needs to be discussed. The data of Table 6 also show that the ratio of total soluble COD uptake to total soluble phosphorus uptake (COD:P) was approximately the same for all units, and averages 81:1. It should be pointed out here, that in these systems that the portion of the soluble COD that is removed from solution is representative of the BOD of the substrate, i.e., that fraction of the total COD that the microorganisms present in the system are able to degrade biologically. Thus it may be stated that in these systems the ratio of BOD uptake to phosphorus uptake (BOD:P) is essentially constant, and averages 81:1. This observation has a definite bearing on the question of whether or not activated sludge systems are able to effect "luxury" uptake of soluble phosphorus.

TABLE 6

UPTAKE OF SOLUBLE PHOSPHORUS AND COD:
MLSS CONCENTRATION STUDIES

Unit Number	1	2	3	4	5
Initial MLSS (mg/l)	8000	6000	3000	1500	750
Total Uptake of Soluble Phosphorus (mg/l as P_2O_5)	82.0	82.5	84.5	82.2	84.5
(mg/l as P)	35.2	35.4	36.3	35.3	36.3
Total Uptake of COD (mg/l)	2925	2850	2825	2860	2875
COD Uptake:P Uptake	83	81	78	81	79
Net Increase in MLSS (mg/l)	1400	1450	1625	1500	1550
MLSS Increase:P Uptake	39.8	40.9	44.6	42.5	42.6

A number of investigators have reported on the BOD:P ratio necessary for the satisfactory operation of activated sludge systems (35, 58, 60, 61, 62). A summary of these findings is shown in Table 7. In general, these reported ratios are based on the minimum amount of phosphorus needed for satisfactory stabilization of the BOD. Thus, these ratios may be thought of as defining the critical amount of phosphorus required for stabilization of the BOD.

When the BOD:P ratio of 81:1 obtained in this study is compared with the values reported by these other investigators, it appears that the activated sludge systems used in the present investigation may have been taking up more phosphorus than was required for the most efficient stabilization of the substrate. If such a comparison is valid, it would tend to support the existence of a "luxury" uptake of phosphorus by activated sludge, i.e., phosphorus uptake greater than that actually required for satisfactory activated sludge operation, as has been reported by others (12, 14).

D. Influence of Initial Substrate Concentration

Previous investigators (12, 14, 21) have reported that the introduction of carbohydrates to activated sludge mixed liquor stimulates the uptake of soluble phosphorus. In addition, the results presented earlier in this report have indicated that connection between the biosynthetic activity of the activated sludge microorganisms, and their uptake of soluble phosphorus. In the light of this information, it seemed desirable to study the influence of initial substrate concentration (COD_i) on the uptake of soluble phosphorus by mixed liquor.

TABLE 7

SUMMARY OF RECOMMENDED BOD:P RATIOS FOR TREATMENT OF WASTES BY ACTIVATED SLUDGE

Source	Recommended BOD:P Ratio	Remarks
Greenberg *et al.* (35)	238:1	Minimal requirement for stabilization of waste.
Eckenfelder (58)	100:1	Recommended value for satisfactory operation.
Sawyer (60)	150:1	Minimal requirement for stabilization of wastes.
Helmers *et al.* (61)	100:1	Minimal requirements for stabilization of waste--20°C.
Helmers *et al.* (62)	143:1	Minimal amount for stabilization of waste--20°C.

Four activated sludge units were initiated for this study, and acclimated to separate initial substrate concentrations. These units were operated in the same manner described earlier for the stock unit, except that each unit received a different amount of substrate (as COD), and the mixed liquor in each unit was wasted differently so as to maintain the same initial MLSS concentration in each unit. Table 8 gives the substrate concentration, wasting schedule, and other pertinent parameters for each of these units. Table 8 also shows that as the COD_i was varied, the phosphorus concentration was also varied, so as to maintain a COD:P ratio of approximately 75:1 in the feed solution. All other nutrient concentrations and operating parameters for these units were the same as those given in Table 1.

Typical data obtained during this phase of the study are presented in Figure 13. It is apparent from these data that increasing the initial substrate concentration has a marked effect on the uptake of soluble phosphorus by activated sludge.

Figure 14 is a presentation of some of the data given in Figure 13, showing the ultimate, i.e., 24-hour, soluble phosphorus uptake plotted against the initial substrate concentration. From inspection of these data, it is apparent that soluble phosphorus uptake is directly proportional to organic loading, provided that aeration is continued until phosphorus uptake ceases.

It was assumed that the soluble phosphorus uptake observed in these systems might more properly be a function of substrate utilization rather than substrate loading. For this reason, the 24-hour soluble phosphorus uptake was plotted against the 24-hour uptake of soluble COD. These

TABLE 8

SUBSTRATE AND NUTRIENT CONCENTRATION AND OTHER OPERATING PARAMETERS: INITIAL SUBSTRATE CONCENTRATION STUDIES

Unit Number	1	2	3	4
Initial Substrate Concentration (mg/l as COD)				
a) glucose	400	600	1200	2400
b) yeast extract	100	150	300	600
c) total	500	750	1500	3000
KH_2PO_4 (mg/l)	11	17	34	68
$Na_2HPO_4 \cdot 7\ H_2O$ (mg/l)	22	33	67	134
Wasting Schedule (% of mixed liquor/day)	14	20	33	50
Initial MLSS Concentration	1500	1500	1500	1500
Initial F:M	0.33	0.50	1.00	2.00
Initial COD:P Ratio	75:1	75:1	75:1	75:1

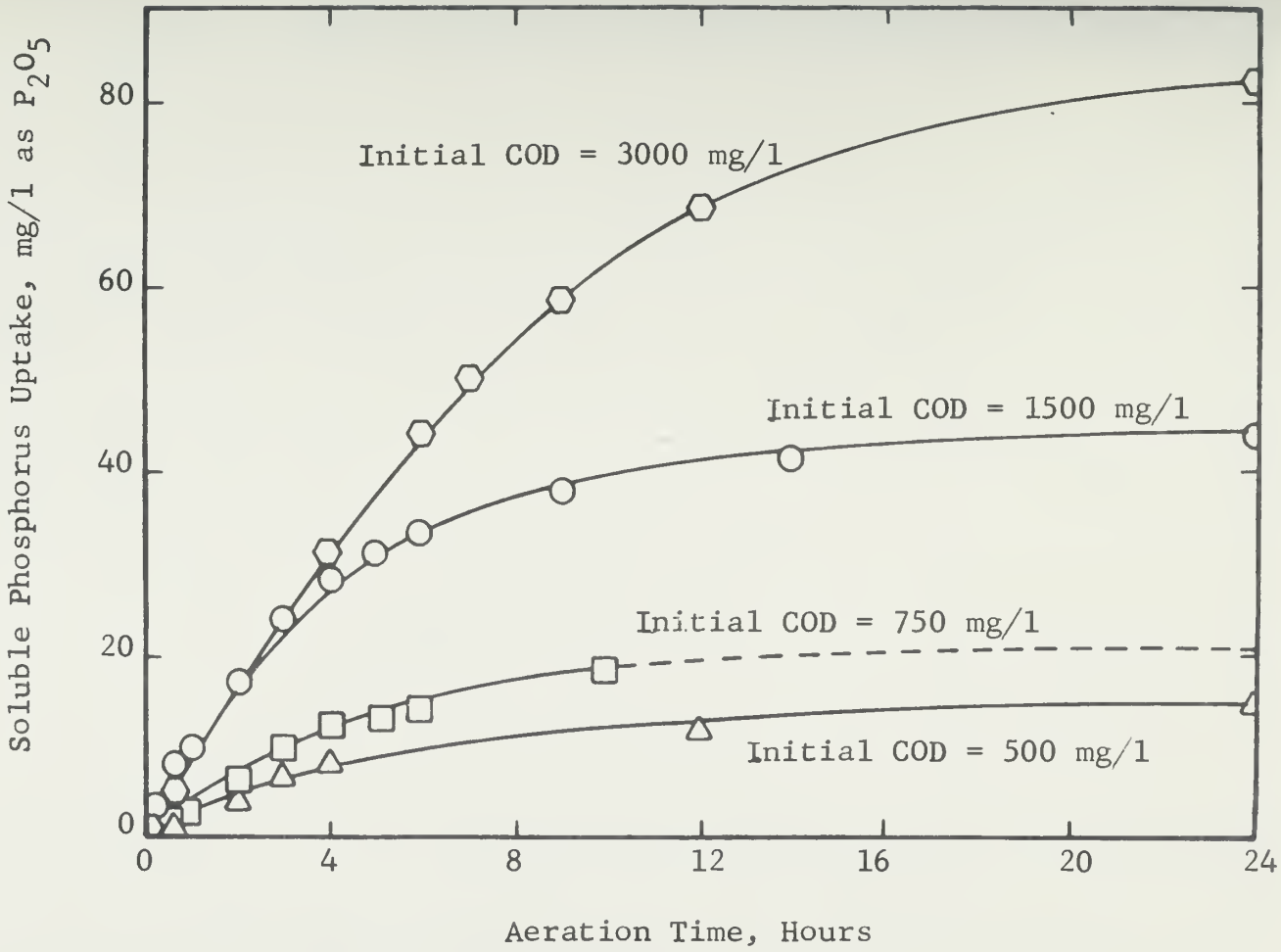

FIGURE 13 INFLUENCE OF INITIAL SUBSTRATE CONCENTRATION ON UPTAKE OF SOLUBLE PHOSPHORUS.

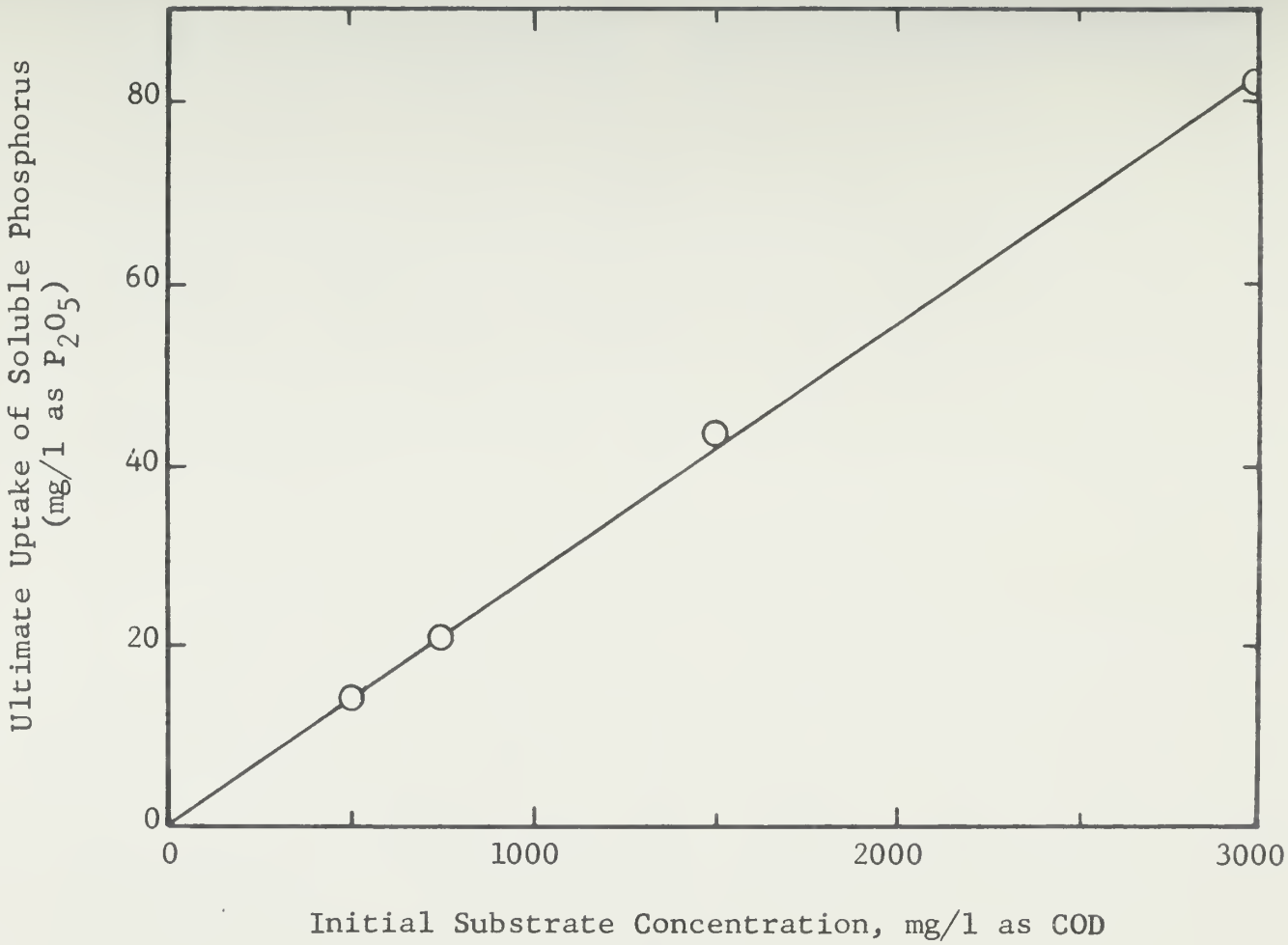

FIGURE 14 PHOSPHORUS UPTAKE AS A FUNCTION OF INITIAL SUBSTRATE CONCENTRATION.

data are shown in Figure 15. From these results the rate of phosphorus removal may be expressed as a function of total COD removal. This expression is as follows:

Soluble Phosphorus Removal Rate = 0.0129 lbs P/(lb COD)(day)

The corresponding ratio of COD uptake to phosphorus uptake is approximately 78:1, very nearly the same as that observed in the MLSS concentration studies discussed earlier. Again, if this value is compared to the BOD:P ratios recommended by other investigators (35, 58, 60, 61, 62) as necessary for satisfactory operation of the activated sludge process (see Table 7), it appears that the activated sludges used in this investigation were taking up phosphorus in greater amounts than was necessary for successful stabilization of the substrate. Thus, these data also support the "luxury" uptake theory advanced by other investigators (12, 14).

Another interesting feature of the ratio of COD uptake to phosphorus uptake obtained in this investigation is its constancy. Regardless of the F:M ratio, and regardless of whether the F:M ratio was varied by holding the substrate concentration constant and varying the MLSS concentration, or vice versa, these systems took up a constant amount of soluble phosphorus for each unit of COD that was utilized. This observation leads to the conclusion that within the limits of this investigation, the uptake of soluble phosphorus in activated sludge systems is directly proportional to the utilization of substrate, i.e., the anabolic activity of the microorganisms present in the system.

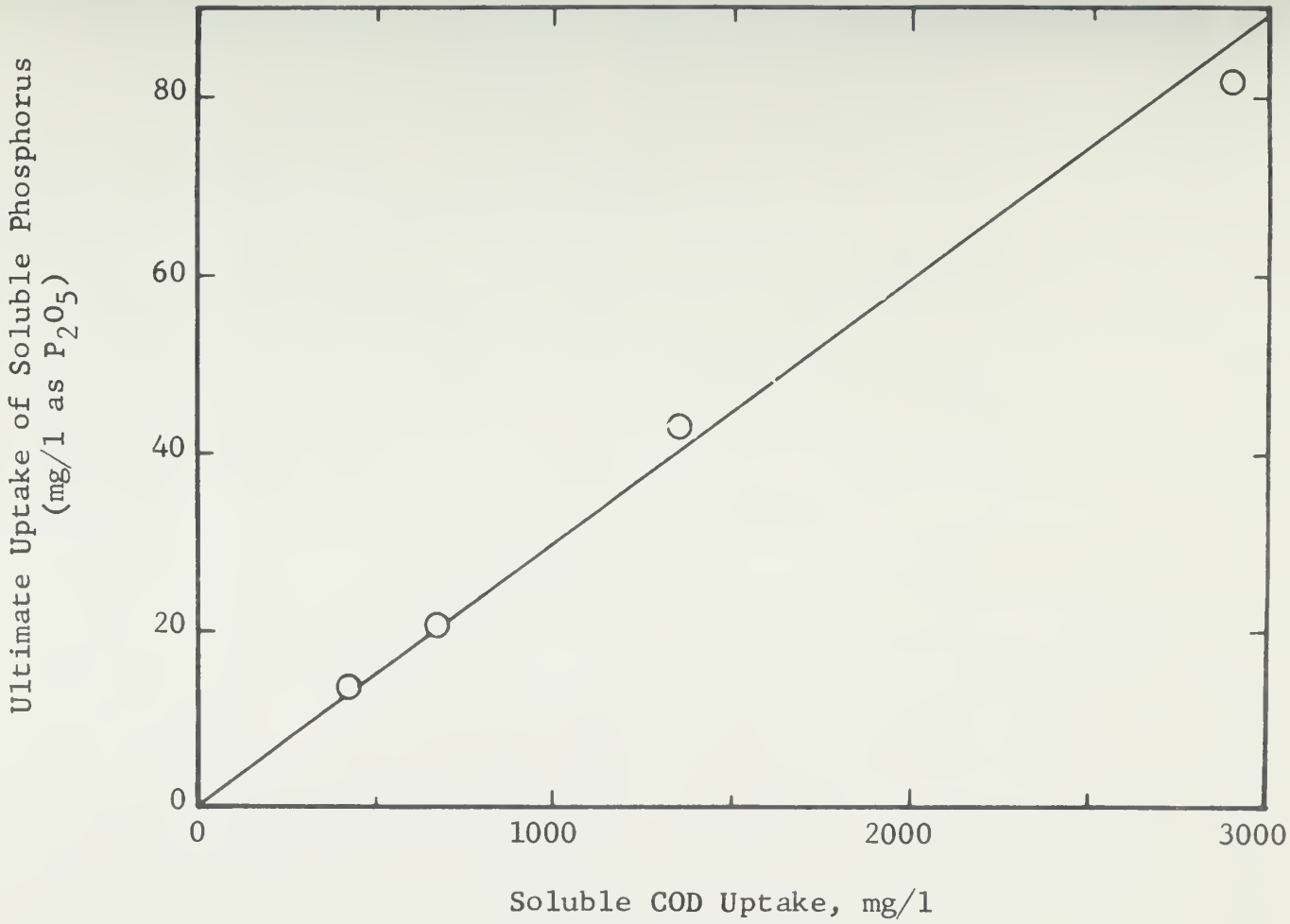

FIGURE 15 SOLUBLE PHOSPHORUS UPTAKE AS A FUNCTION OF COD UPTAKE.

The data reported by Jenkins (63) may also be used to support this conclusion. Working with continuous flow, pilot scale activated sludge units operated at loading rates varying from 0.1 to 3.0 pounds of COD removed per pound of volatile suspended solids per day (lb COD removed/lb vss-day), Jenkins (63) was able to show that the ratio of phosphorus removed to COD removed, at all loading rates, was a constant value. He reported this value to be approximately 0.0086 pounds of phosphorus removed per pound of COD removed. It is obvious that this value cannot be compared directly with the ratio of phosphorus uptake to COD uptake obtained from this investigation, because of the differences in the type of operation and in the units of expression. Nevertheless, Jenkins' data do support the conclusion that this ratio is constant over a wide range of activated sludge loading rates, and that the uptake of soluble phosphorus in activated sludge systems is related to the system's anabolic utilization of substrate.

Additional data obtained during this phase of the study are presented in Figure 16, which shows the total 24-hour uptake of soluble phosphorus plotted versus the increase in MLSS concentration that occurred during this period. The slope of the curve shown in Figure 16 can be used to compute the ratio of net MLSS increase to total phosphorus uptake (MLSS increase:P uptake) in these systems. As computed from these data, this ratio has a value of 42.5:1, a value almost identical to that which was obtained from the MLSS concentration studies discussed earlier (Chapter V, Section C). Thus, within the limits of this investigation, it was concluded that regardless of the F:M ratio, and regardless of the

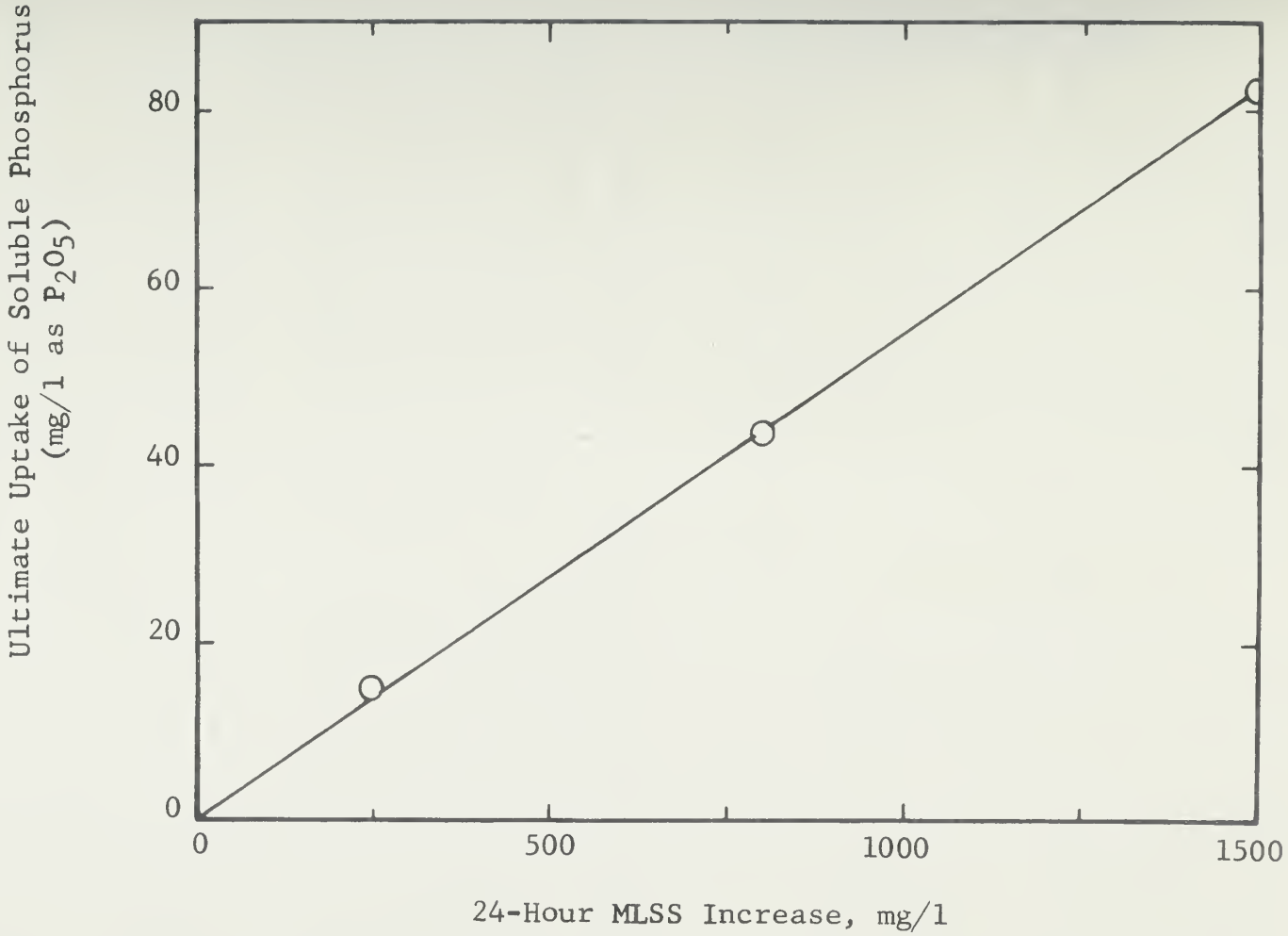

FIGURE 16 SOLUBLE PHOSPHORUS UPTAKE AS A FUNCTION OF MLSS SYNTHESIS

manner in which the F:M ratio is controlled, the amount of phosphorus taken up by the activated sludge is directly proportional to the system's metabolic synthesis.

The data on phosphorus uptake in activated sludge reported by Jenkins (63) also support this conclusion. Jenkins (63) was able to show, using continuous flow, pilot scale activated sludge units operated at loading rates varying from approximately 0.3 to 43 pounds of COD removed per pound of volatile suspended solids per day, that the phosphorus content of the volatile sludge solids, on a dry weight basis, did not vary significantly from a value of 2.6 percent over the entire range of organic loading. This value is quite comparable to the ratio of phosphorus uptake to MLSS increase observed in this study, i.e.,

$$\text{P uptake:MLSS increase} \cong 1:42 \cong .024 \cong 2.4\%$$

These observations imply that a constant amount of phosphorus is required for each unit of volatile suspended matter that is synthesized, i.e., that the uptake of phosphorus in activated sludge systems is directly proportional to the net synthesis that occurs. These results offer further proof of the connection between metabolic activity, i.e., cellular synthesis, and the uptake of soluble phosphorus by activated sludge systems.

As shown in Table 8, the initial COD:P ratio in these units was held constant. This means that the initial soluble phosphorus concentration (SOP_i) of these systems was directly proportional to the initial substrate concentration. It was possible therefore to attribute the observed increase in soluble phosphorus uptake to the increase in

SOP_i rather than to the increase in COD_i. For this reason, a series of experiments were performed using the same activated sludge units described earlier in Table 8 with the exception that each unit now received the same initial soluble phosphorus concentration. It was reasoned that if the difference in soluble phosphorus removal shown in Figures 13, 14, 15, and 16 was affected by the initial soluble phosphorus concentration rather than by the initial substrate concentration, the amount of soluble phosphorus removed in these systems should be equal.

Typical results of these experiments are presented in Figure 17. For this particular set of data, the initial soluble phosphorus concentration in each of the three units was 45 mg/l as P_2O_5. Although these results do not rule out a relationship between the systems' initial soluble phosphorus concentration and their removal of soluble phosphorus, they do show that the uptake of soluble phosphorus is strongly influenced by the initial substrate concentration.

E. Influence of Dissolved Oxygen Concentration

Rudolfs (23), in 1947, noted the influence of dissolved oxygen on the soluble phosphorus content of mixed liquor. He reported a release of soluble phosphorus from sludge which had become anaerobic. Campbell (26) has reported that return sludge, kept under anaerobic conditions at room temperature for four hours, leaked phosphorus from the sludge solids into the liquid.

In 1961, Alarcon (22) demonstrated that the dissolved phosphorus content of activated sludge mixed liquor decreased during aeration, and increased when aeration was stopped. Shapiro (64) has recently reported

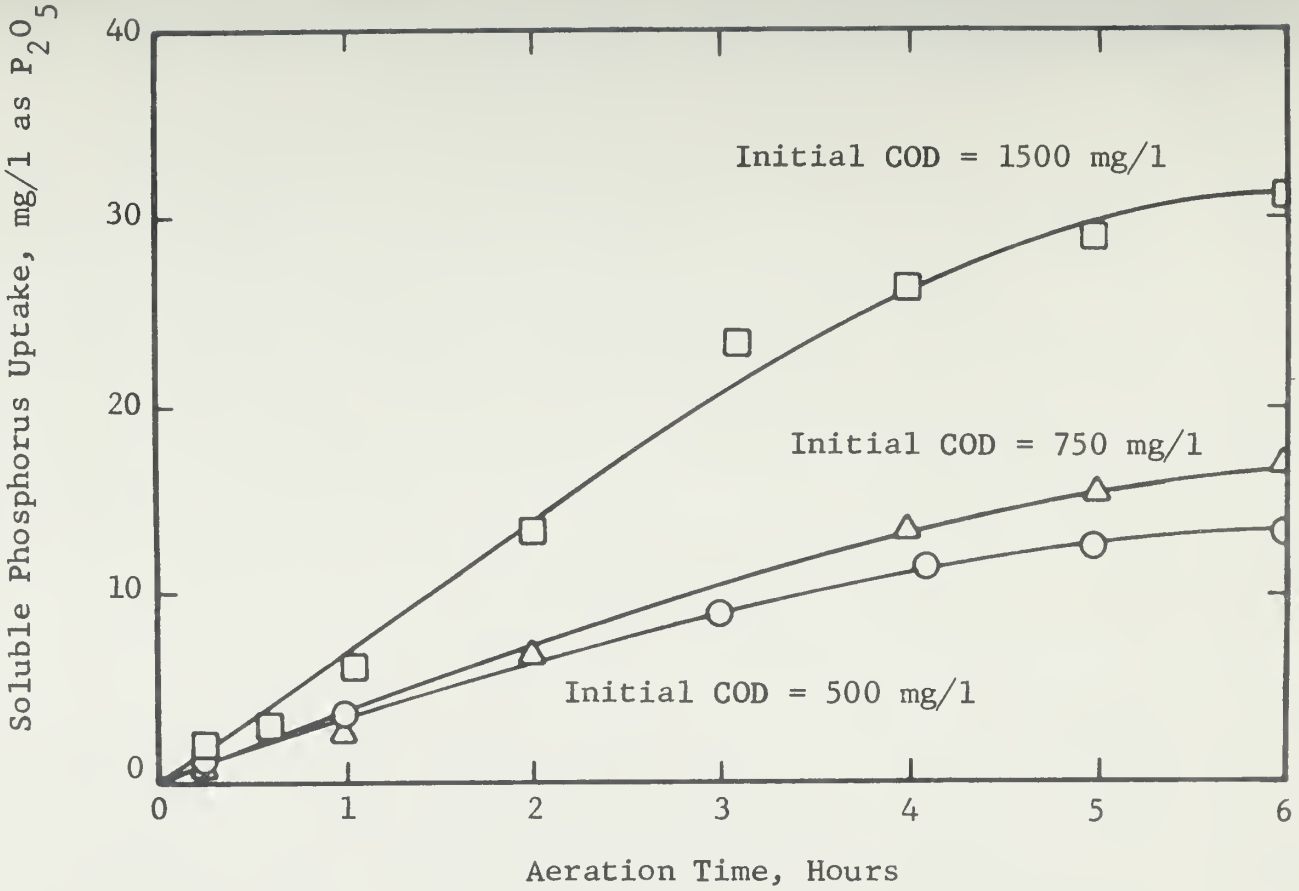

FIGURE 17 UPTAKE OF SOLUBLE PHOSPHORUS AT VARIOUS INITIAL SUBSTRATE CONCENTRATIONS - CONSTANT INITIAL SOLUBLE PHOSPHORUS CONCENTRATION, 45 mg/l as P_2O_5.

on similar observations. Using both activated sludge and dense cultures of Escherichia coli, he was able to demonstrate that the microorganisms were able to release a large proportion of their phosphorus to the medium in a matter of hours when kept under anoxic conditions. Shapiro (64) was also able to show that this release of phosphorus was reversible, with the microorganisms taking up the phosphorus again when aeration was resumed.

Levin and Shapiro (12) found soluble phosphorus uptake to be a function of aeration rate, rather than the total quantity of air supplied, up to an aeration rate of 680 ml/l/min. At an aeration rate of 40 ml/l/min, which they reported as typical of actual plant operation, there was a release of phosphorus into solution. In a system that was not aerated there was also a leakage of phosphorus from the sludge back into solution. Unfortunately, no dissolved oxygen data were presented for these experiments.

Levin and Shapiro (12) confirmed their laboratory observations that an increased rate of aeration improved phosphorus removal, by conducting similar plant scale studies at the District of Columbia Sewage Treatment Plant. Results indicated that more phosphorus was removed in an aeration basin operated at 3 to 4 times the aeration rate used in the plant than in the control basin aerated at the normal rate. They reported that the DO level in the experimental basin was much higher than in the control basin, which often had a DO concentration of zero. From these data, however, it is difficult to evaluate the absolute effect and overall significance of either aeration rate or DO concentration on phosphorus uptake.

Consequently, it was decided that further investigations in this area were in order.

The initial experiments in this series were conducted in the following manner. Experimental activated sludge units were started as described previously. However, after 6 hours aeration, the contents of these units were divided into three aliquots. One of these aliquots continued to be aerated as before, at an air flow rate of 4 $ft^3/(hr)(l)$. A high DO concentration was maintained in this aliquot throughout the experiment. A second aliquot was allowed to settle quietly. This aliquot was representative of a secondary settling basin which receives effluent from an aeration tank with a 6-hour detention period. The DO concentration in this aliquot went rapidly to zero. The third aliquot was continuously mixed by supplying it with nitrogen gas at a rate equal to the original air flow rate. The DO concentration of this aliquot was also zero. This aliquot was used in an effort to gain additional insight into the effects of mixing and dissolved oxygen concentration on the soluble phosphorus content of these systems.

The influence of dissolved oxygen on the uptake of soluble phosphorus by these systems is readily apparent from the data of Figure 18. In the aliquot maintained at the high DO level there was continuous removal of dissolved phosphorus throughout the aeration period. On the other hand, the two aliquots that were subjected to conditions of zero DO ceased removing phosphorus from solution almost immediately.

These data also indicate that turbulent mixing is not an important parameter in the uptake of soluble phosphorus by these systems. In the aliquot which received nitrogen gas instead of air, there was no

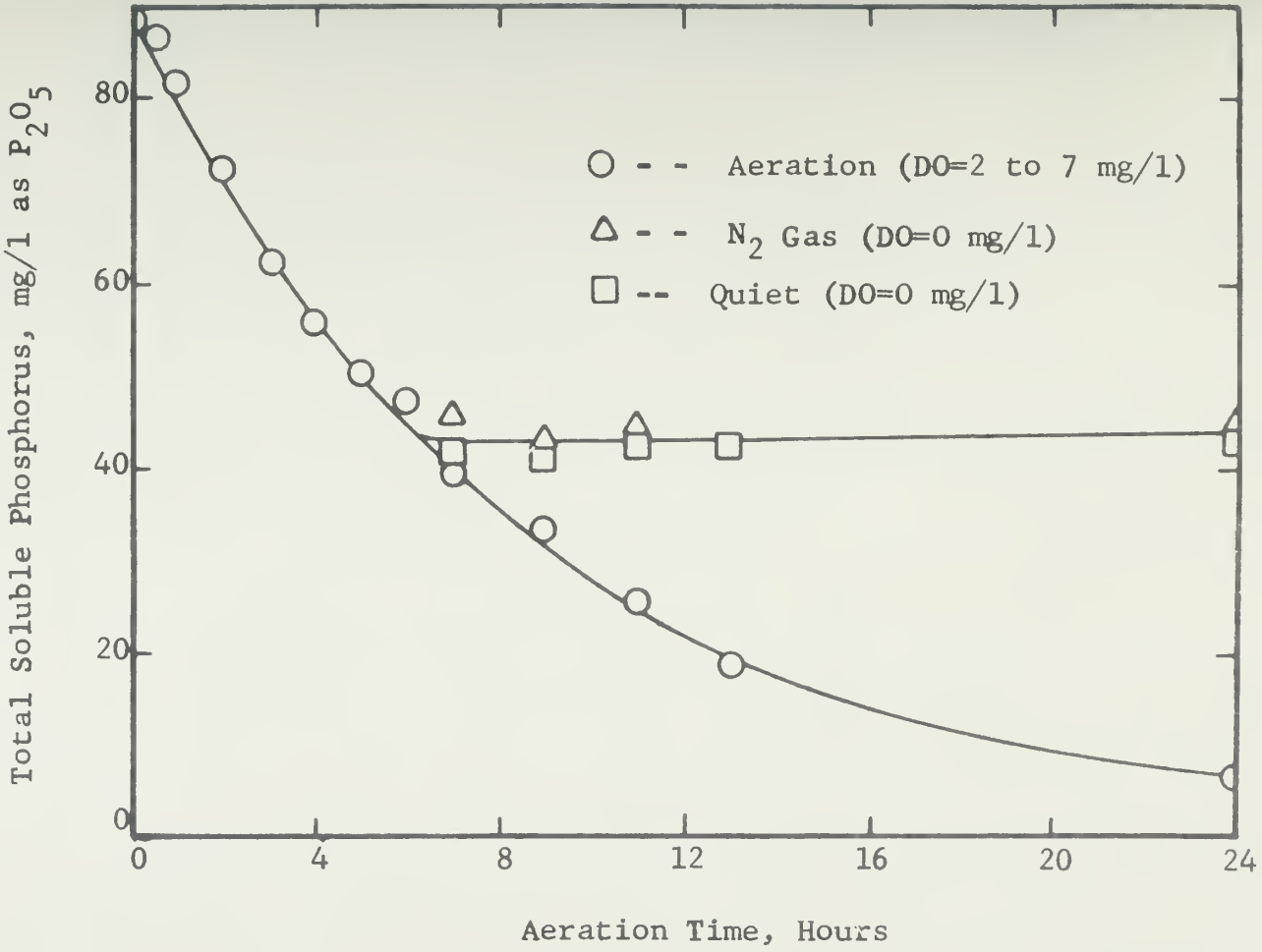

FIGURE 18 SOLUBLE PHOSPHORUS UPTAKE, SYSTEM RESPONSE TO CONTINUOUS AERATION, MIXING WITH N_2 AND QUIET SETTLING.

further removal of soluble phosphorus once the DO concentration reached zero, even though the total gas flow rate, and therefore the degree of mixing, was the same as for the continuously aerated aliquot.

The data of Figure 18 also show that there was no significant release of soluble phosphorus from the solids back into the liquid during the anaerobic period. This was typical of this entire investigation, since phosphorus leakage under conditions of zero DO was never observed in any of these experiments. This fact is apparently contrary to the findings of previous investigators (12, 22, 23, 26, 64). However, these data are supported by the plant scale studies of Vacker et al. (29), who reported that leaking of phosphorus from the solids back into the liquid during final clarification was not significant if a high dissolved oxygen level had been sustained during at least half of the aeration period.

Typical results of experiments designed to study the effect of aeration rate on the uptake of soluble phosphorus are presented in Figure 19. For these investigations two identical experimental activated sludge units were started as described earlier. These units were both operated in an identical manner, except for their rate of aeration. One of these units was aerated at a rate of 4.0 ft^3/(hr)(l) (1888 ml/(min)(l)), the aeration rate normally employed throughout this entire investigation. The second unit was operated at an aeration rate of 0.5 ft^3/(hr)(l) (236 ml/(min)(l)). This latter rate was selected because a low rate was desired for the experiment, and because this particular rate could be easily controlled and measured with the available equipment. Furthermore, rates lower than this would not keep the sludge in suspension.

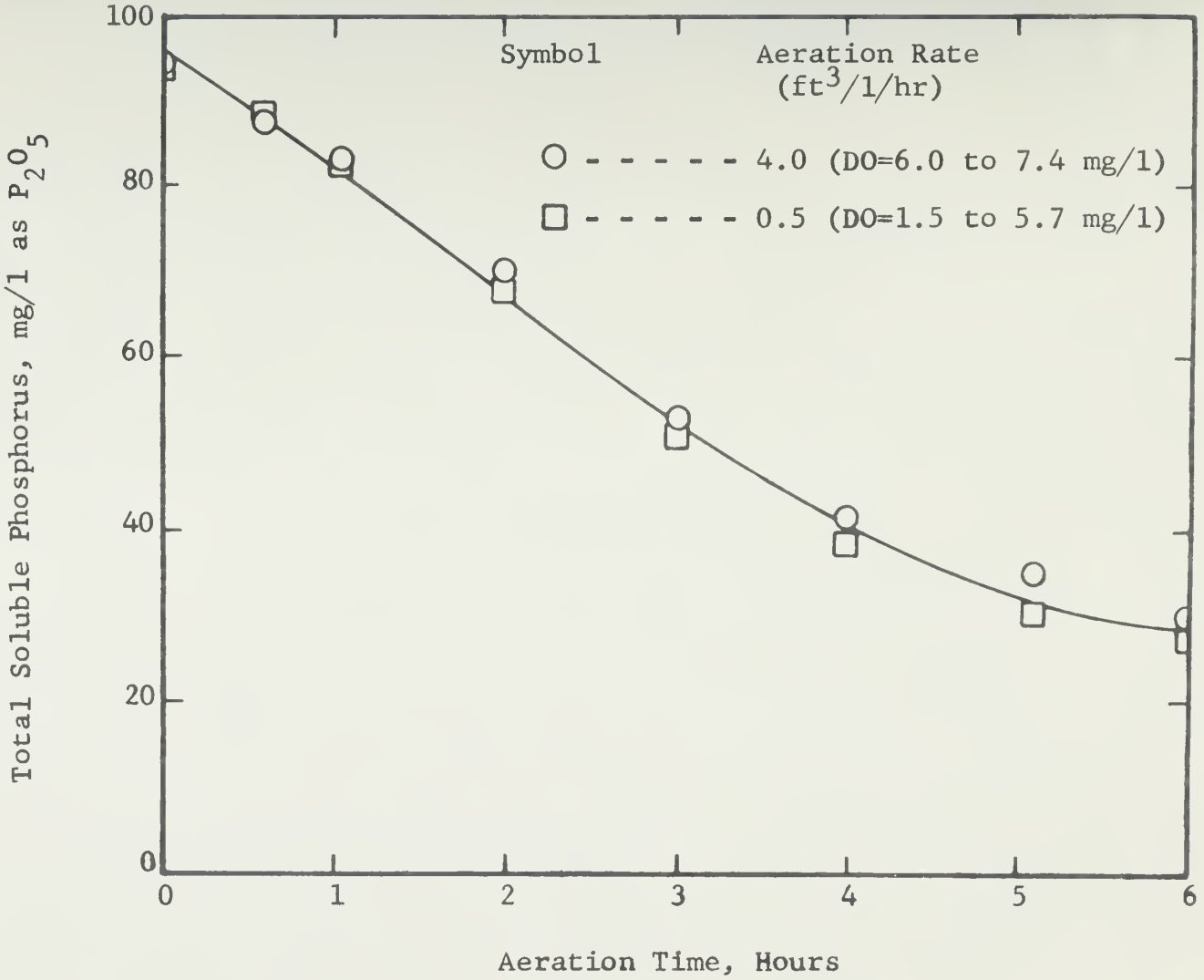

FIGURE 19 SOLUBLE PHOSPHORUS UPTAKE, SYSTEM RESPONSE TO AERATION RATE.

Inspection of the data given in Figure 19 reveals no significant difference in either the rate or extent of soluble phosphorus uptake in the two systems. Within the limits studied, therefore, aeration rate does not appear to be an important parameter in the uptake of soluble phosphorus by these systems.

The data of Figure 19 also show that, although the dissolved oxygen concentration was lowest in the unit operated at the lower aeration rate, it was apparently high enough to enable the unit to function in a normal manner. In other words, DO concentrations of approximately 1.5 mg/l, or greater, do not appear to restrict these systems' uptake of soluble phosphorus. This conclusion will be expanded upon later.

Results of additional experiments performed during this phase of the investigation are shown in Figure 20. In these studies an experimental activated sludge unit was started as described previously. After 6 hours of aeration, the mixed liquor content of the unit was split into two aliquots. One of these aliquots continued to be aerated as before, and the second aliquot was allowed to settle quietly. After 6 hours of quiescent settling, the second aliquot was gently but thoroughly mixed and split into two portions. One of these portions was allowed to settle quietly as before, and the second was aerated at the same rate as originally.

The data presented in Figure 20 have several features of interest to this discussion. For the continuously aerated activated sludge, it may be seen that the removal of COD and the increase in MLSS were quite typical, and were essentially completed by the end of the second hour

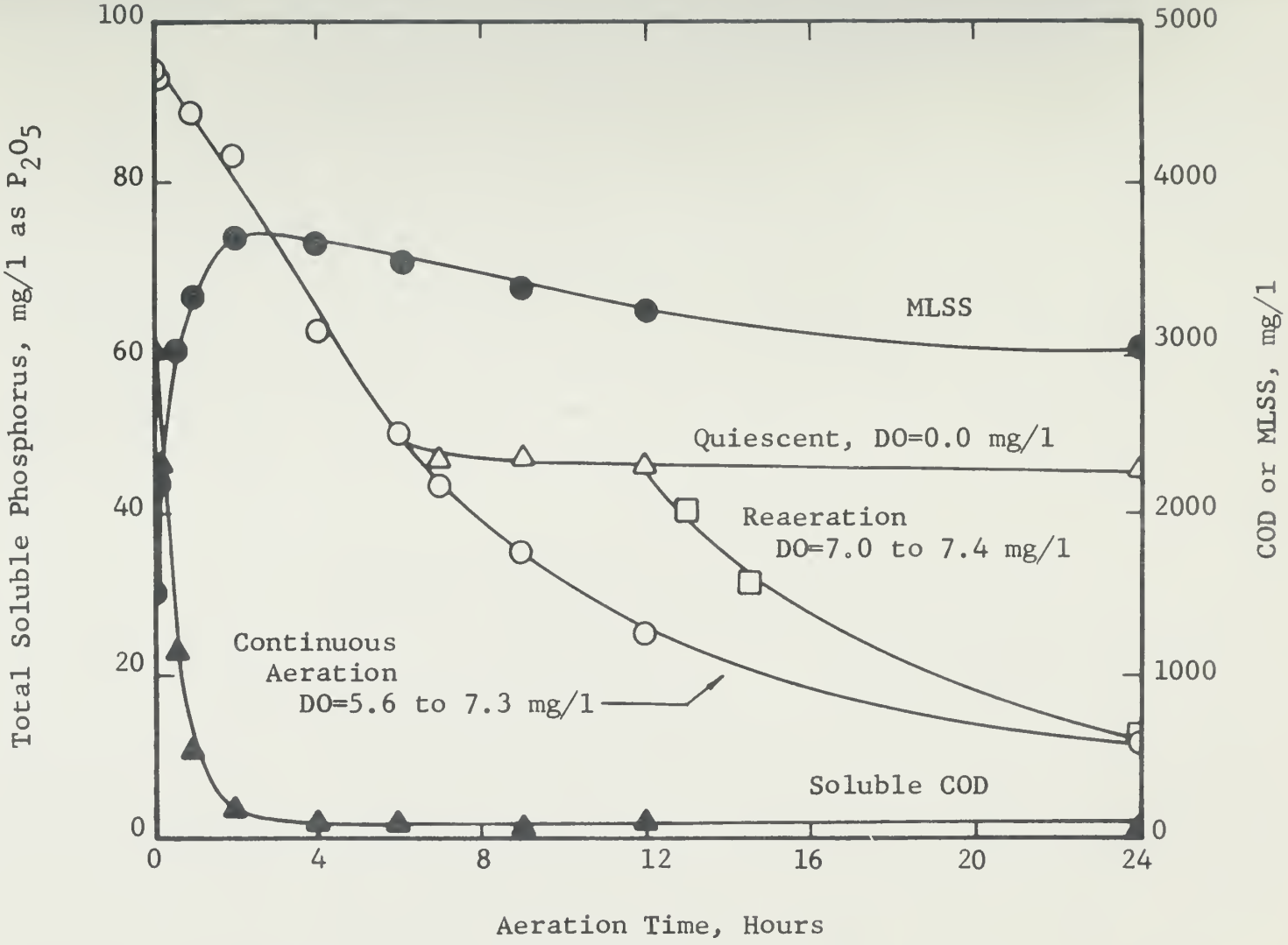

FIGURE 20 PHOSPHORUS UPTAKE AS INFLUENCED BY DISSOLVED OXYGEN

of aeration. The soluble phosphorus concentration, on the other hand, continued to decrease throughout the aeration period. This observation was also made earlier in this report, during the discussion of the data presented in Figure 6. These results indicate that the uptake of soluble phosphorus is more closely associated with the metabolic utilization of substrate than with the more rapid uptake and storage of the substrate.

It should also be noted that when aeration was stopped, the DO concentration of the mixed liquor rapidly went to zero, and phosphorus uptake ceased. This was also noted earlier, and was expected since, in the absence of DO the aerobic microorganisms present in the system would be incapable of aerobic metabolism, and the facultative microorganisms would be forced to shift from aerobic to anaerobic metabolism. It is well established that the anaerobic metabolism of carbohydrates yields less energy for synthesis, and requires less inorganic phosphorus than does aerobic metabolism. Consequently, the uptake of soluble phosphorus should be drastically curtailed in the absence of DO. This hypothesis is supported by the data of Figure 20, which show an immediate cessation of phosphorus uptake when aeration was stopped. Furthermore, when aeration was resumed, and aerobic metabolism was once again possible, phosphorus uptake was immediately resumed.

Figure 21 presents the results of experiments designed to study the effect of various mixed liquor DO concentrations on the uptake of soluble phosphorus. These data were obtained from identical activated sludge systems operated under different conditions of aeration. Nitrogen, air, and a mixture of the two were employed in such a way that the turbulent

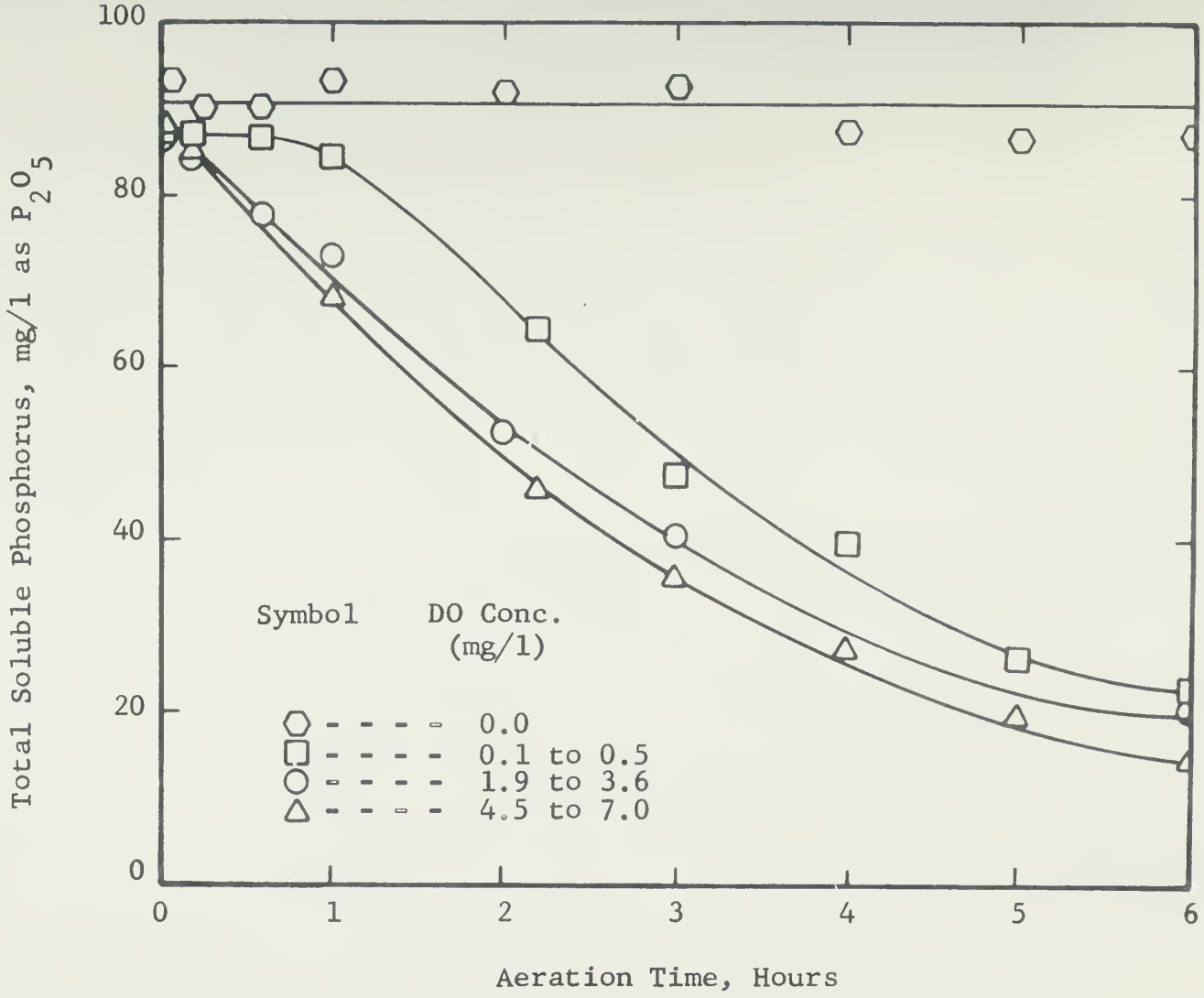

FIGURE 21 SOLUBLE PHOSPHORUS UPTAKE AS A FUNCTION OF DISSOLVED OXYGEN CONCENTRATION.

mixing of the systems was identical, but so that the DO concentrations were different. The total gas flow rate was the same in all cases.

From these data it can be concluded that the uptake of soluble phosphorus is not directly related to aeration rate, i.e., total gas flow rate. On the other hand, the DO concentration of the system exerts a marked effect on the systems' response in terms of phosphorus uptake. Under anaerobic conditions, no soluble phosphorus was taken up. Phosphorus uptake did occur in systems operated at low DO levels, i.e., 0.1 to 0.5 mg/l, but was preceded by a rather pronounced lag period. At the higher DO levels (1.9 to 7.0 mg/l) phosphorus uptake began immediately.

These observations indicate that sustained aeration tank DO levels on the order of 2 mg/l are necessary for the optimum uptake of soluble phosphorus by activated sludge systems. Furthermore, the long lag period in phosphorus removal associated with DO levels less than approximately 2 mg/l, indicates that phosphorus uptake may be severely restricted in activated sludge plants in which the DO concentration along a major portion of the aeration tank is less than this optimum value. This conclusion is also supported by the data of Vacker et al. (29) who noted that very little soluble phosphorus uptake occurred until the aeration tank DO level approached or exceeded 2 mg/l.

F. Influence of pH

Levin and Shapiro (12) investigated the effect of pH on the uptake of orthophosphate by activated sludge. They reported that the pH of the mixed liquor had a significant influence on orthophosphate uptake, with a maximum uptake occurring in the pH range 7.0 to 8.0. However,

the sludge used in their experiments was not acclimated to the pH levels being studied. For this reason it seemed desirable to evaluate the influence of pH on the uptake of soluble phosphorus by activated sludges acclimated to various pH levels. The results of studies performed on systems acclimated to pH values between 6.0 and 9.0 are presented in Figure 22.

These data show that the rate and extent of soluble phosphorus uptake are markedly reduced in the systems operated at the lower pH levels. Moreover, most of this effect occurs in the pH range between 7.5 and 6.5, and there is little further reduction in uptake capacity as the pH is lowered to 6.0. These results are in general agreement with those of Levin and Shapiro (12); however, with the acclimated sludges used in the present study, systems operated at pH 9.0 appeared to be as effective for the removal of soluble phosphorus as those operated at 7.5.

G. Influence of Initial Soluble Phosphorus Concentration

Prior to this phase of the investigation, practically all of the experimental work had been performed with activated sludge units in which the initial COD:P ratio was very nearly the same. As mentioned earlier, this value was approximately 75:1, a value selected to insure the satisfactory performance of the activated sludge process. However, in seeking evidence of a "luxury" uptake of soluble phosphorus in these units, it was decided to investigate their operation at other, lower, COD:P ratios. It was reasoned that these systems might take up phosphorus in excess of those amounts already observed, if greater amounts of phosphorus were available.

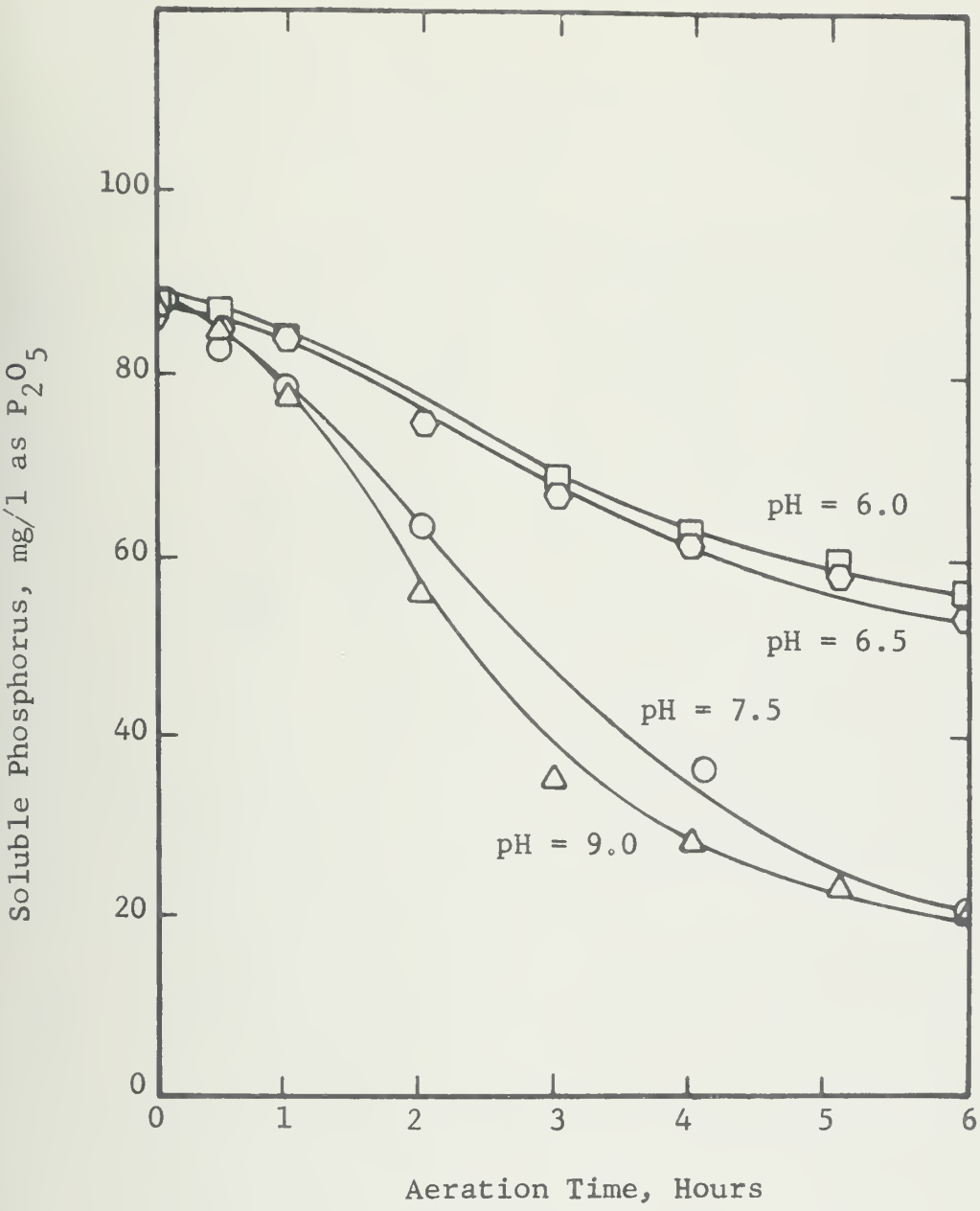

FIGURE 22 SOLUBLE PHOSPHORUS UPTAKE AS A FUNCTION OF pH.

These studies were carried out using activated sludges that were originally developed for the studies on the influence of initial substrate concentration, which are described in Table 8. However, for this phase of the investigation the experimental units were modified slightly, as follows.

The experimental units were prepared in the normal manner, using biological solids, feed solution and water as described earlier. However, before aeration was begun, the contents of the units were split into three aliquots. One of these aliquots remained unaltered, and thus had an initial COD:P ratio of approximately 75:1. The remaining two units each received different additional amounts of the stock phosphorus solution. The effect of this operation was to establish three experimental units, identical in all respects except initial soluble phosphorus (SOP_i) concentration. Aeration was then begun in all three aliquots, and sampling was started. It should be pointed out that the stock phosphorus solution was of sufficient strength to insure against significant dilution of the three systems.

Figure 23 is representative of the results obtained from these studies. These particular data were obtained from systems containing an initial MLSS concentration of 1500 mg/l, which received an initial COD loading of 750 mg/l, giving them an initial F:M of 0.5. The initial COD:P ratio in the three systems was approximately 75:1, 39:1, and 23:1, for the low, medium, and high phosphorus systems, respectively. From these data it can be seen that the initial rate of soluble phosphorus uptake was the same in all three aliquots. The total magnitude of uptake, however, was increased in the systems having higher SOP_i concentrations. This was rather surprising in light of the fact that, in all

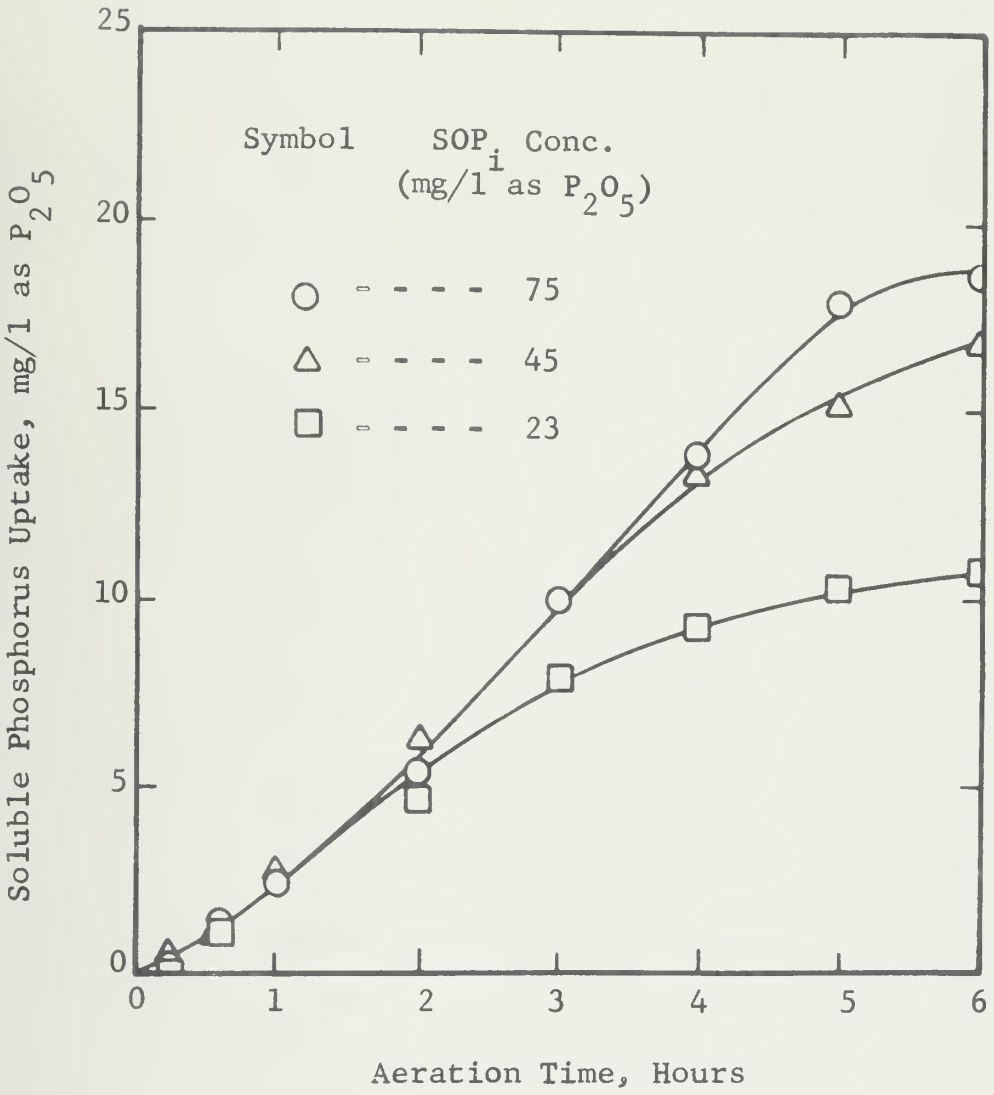

FIGURE 23 SOLUBLE PHOSPHORUS UPTAKE AS A FUNCTION OF INITIAL SOLUBLE PHOSPHORUS CONCENTRATION.

cases, phosphorus was apparently not limiting, there being significant amounts remaining in solution in each of the systems at the end of the aeration period.

These data also reveal that the incremental increase in the 6-hour uptake value was not proportional to the incremental increase in the SOP_i concentration. This fact suggests that the uptake mechanism can be saturated at high SOP_i values.

Figure 24 presents the data on soluble COD uptake and the increase in MLSS concentration obtained from these three systems. Inspection of these data reveals that the uptake of COD and the increase in MLSS was essentially the same in all three aliquots. These observations are quite puzzling when considered in conjunction with results presented earlier in this report. These data, however, do support the contention that, under certain conditions, activated sludge systems may take up soluble phosphorus in amounts greater than that needed for normal cell growth.

These data also indicate that the relationships between COD uptake and phosphorus uptake, and between MLSS increase and phosphorus uptake, which were presented earlier in this discussion, were probably influenced by the amount of soluble phosphorus available to the systems. Therefore, the ratios that were derived and presented earlier, i.e.:

$$\text{COD uptake:P uptake} \cong 80:1$$

$$\text{MLSS increase:P uptake} \cong 42:1$$

are correct only for those systems having an initial COD:P ratio of approximately 75:1. Most probably, these values would be different for systems having initial COD:P ratios other than this.

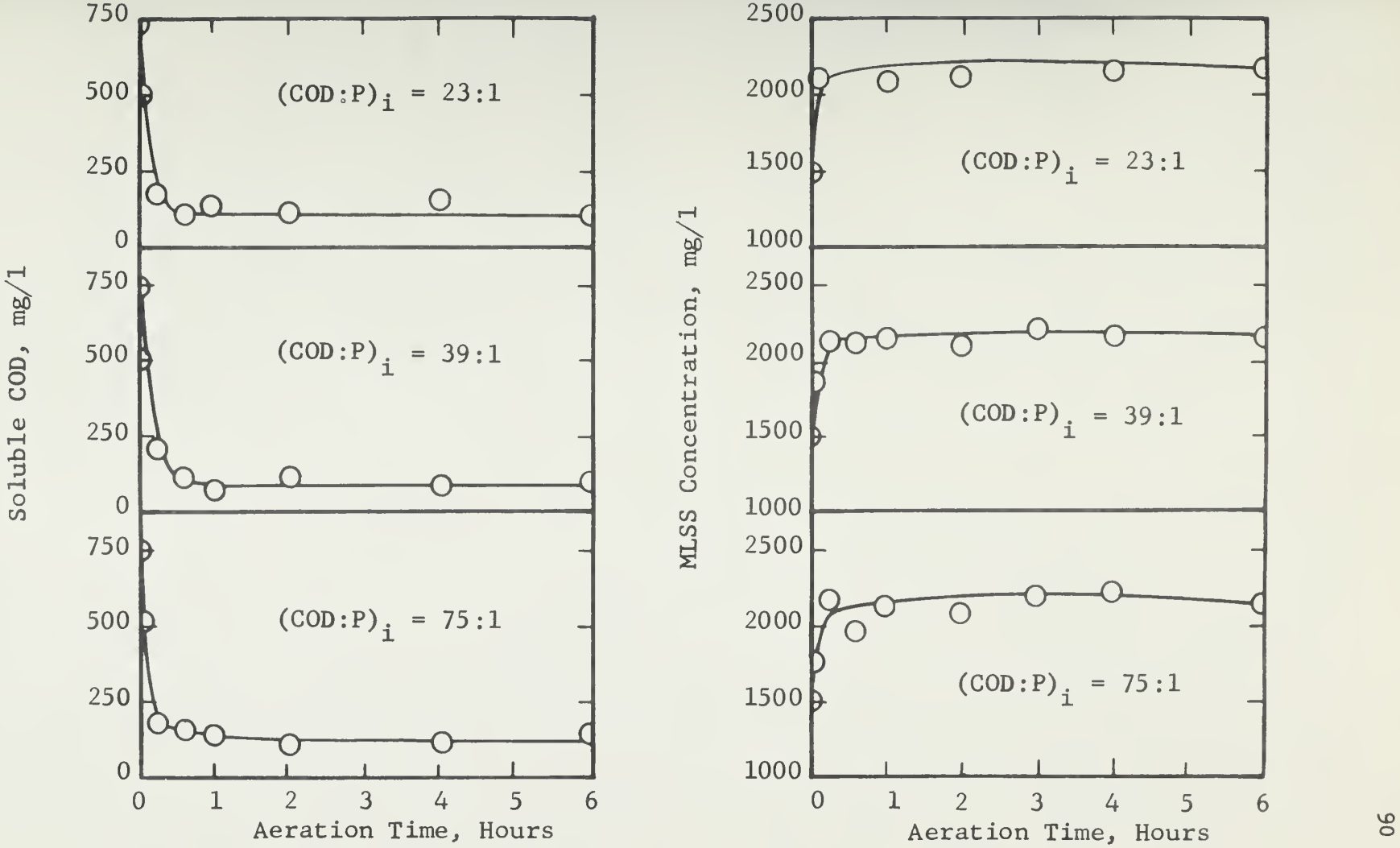

FIGURE 24 VARIATION IN SOLUBLE COD AND MLSS CONCENTRATION DURING AERATION OF ACTIVATED SLUDGE: INITIAL SOLUBLE PHOSPHORUS CONCENTRATION STUDY.

Figure 25 presents further data relative to the influence of initial soluble phosphorus concentration on the uptake of phosphorus by activated sludges. These data show the effect of initial soluble phosphorus concentration on the uptake of soluble phosphorus in activated sludge systems operated at different substrate loading rates. The initial MLSS concentration was 1500 mg/l in all cases. The initial COD concentration is shown on each curve. It may be seen that for each of the loading rates studied an increase in the system's initial soluble phosphorus concentration resulted in an increase in the system's uptake of soluble phosphorus. These data indicate that both substrate loading and initial soluble phosphorus concentration greatly influence the total amount of soluble phosphorus that can be taken up by activated sludge systems.

In order to gain a better understanding of this phenomenon, fractionation studies were performed on the microorganisms composing the activated sludge. For this study only two aliquots were used, one containing a normal initial concentration of soluble phosphorus (initial COD:P = 75:1), and one containing a high initial concentration of soluble phosphorus (initial COD:P = 40). The purpose of this study was to compare the various cellular fractions under conditions of high and low SOP_i concentration, in the hope of discovering the cellular fraction or fractions which accounted for the excess phosphorus.

Data from this study are presented in Table 9. Inspection of these data shows that the system containing the higher SOP_i concentration was able to take up considerably more soluble phosphorus (approximately 60

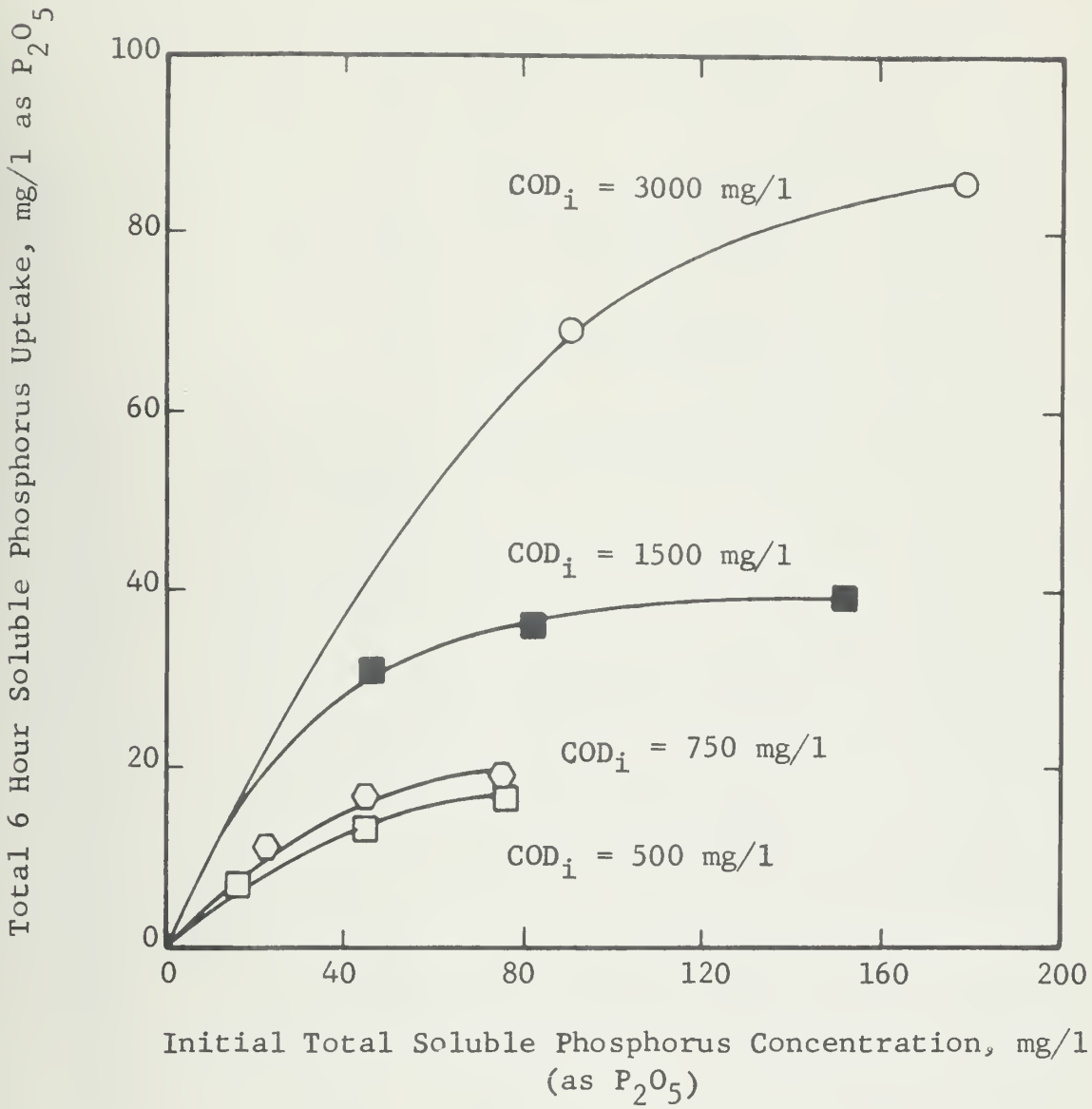

FIGURE 25 INFLUENCE OF INITIAL SOLUBLE PHOSPHORUS CONCENTRATION ON TOTAL 6 HOUR UPTAKE OF SOLUBLE PHOSPHORUS BY ACTIVATED SLUDGE SYSTEMS OPERATED AT DIFFERENT LOADING RATES.

TABLE 9

CELL FRACTIONATION STUDIES, INFLUENCE OF INITIAL SOLUBLE PHOSPHORUS CONCENTRATION ON SOLUBLE PHOSPHORUS UPTAKE

Time (min)	Phosphorus Concentration (mg/l as P_2O_5)											
	Total Uptake		Total Insoluble									
			0.85% NaCl Wash		Metabolic Intermediates (Cold TCA)		Phospholipids (Hot ETOH)		Nucleic Acids (Hot TCA)		Protein (Residue)	
	High[a]	Low[b]	High	Low	High	Low	High	Low	High	Low	High	Low
3	0.0	0.0	0.7	0.6	21.2	22.5	2.2	2.5	45.0	48.4	2.9	2.6
30	3.5	6.5	0.7	0.7	26.3	27.0	4.2	3.1	45.0	50.0	2.9	4.3
60	24.0	11.0	3.0	1.3	25.2	26.5	4.7	4.1	53.0	57.4	3.4	5.1
120	42.0	26.5	1.3	0.5	26.6		9.2	7.3	56.0	56.0	2.9	4.5
246	72.5	53.0	1.3	1.3	23.2	23.7	5.7	8.0	86.0	75.5	4.7	4.4
360	85.5	69.6	1.2	0.5	31.4	23.0	3.6	4.0	111.0	101.0	5.3	5.7
660	118.0	83.7	0.9	0.3	34.0	31.4	5.1	4.2	139.0	115.0	5.8	5.7
1440	149.0	89.3	1.5	0.3	46.6	34.5	4.9	6.7	162.0	118.0	10.5	7.9

[a]High = SOP_i of 179 mg/l as P_2O_5.

[b]Low = SOP_i of 90 mg/l as P_2O_5.

mg/l) than the low SOP_i system. A close examination of these data shows that the only significant difference in the phosphorus content of the various fractions at the high and low SOP_i concentrations is found in the two acid-soluble fractions, i.e., the metabolic intermediates and other low molecular weight compounds, and the nucleic acids. The data for these two fractions are shown in Figure 26. The data of Table 9 and Figure 26 show that these two fractions account for more than 90 percent of the additional phosphorus taken up by the high SOP_i system, with approximately three-fourths of this going into the nucleic acid fraction. These data seem to indicate that this uptake of excess phosphorus was also closely related to the cells' anabolic activity. However, the reason for this excess or "luxury" uptake is not understood at the present time.

Table 10 is a summary of selected data from Table 9, showing the phosphorus content of the various cellular fractions as a percent of each system's total insoluble phosphorus. These data show that essentially all of the phosphorus removed from solution by both the high and the low SOP_i system was recoverable in the various cellular fractions. These figures show that an average of 108.9 and 97.8 percent of the soluble phosphorus taken up by the high and low SOP_i systems, respectively, was recoverable in the cellular fractions. This observation again points to the dominant role that the microorganisms play in the uptake of soluble phosphorus by these systems.

Although not shown here, other data obtained during the course of this experiment revealed that the uptake of soluble COD, and the net

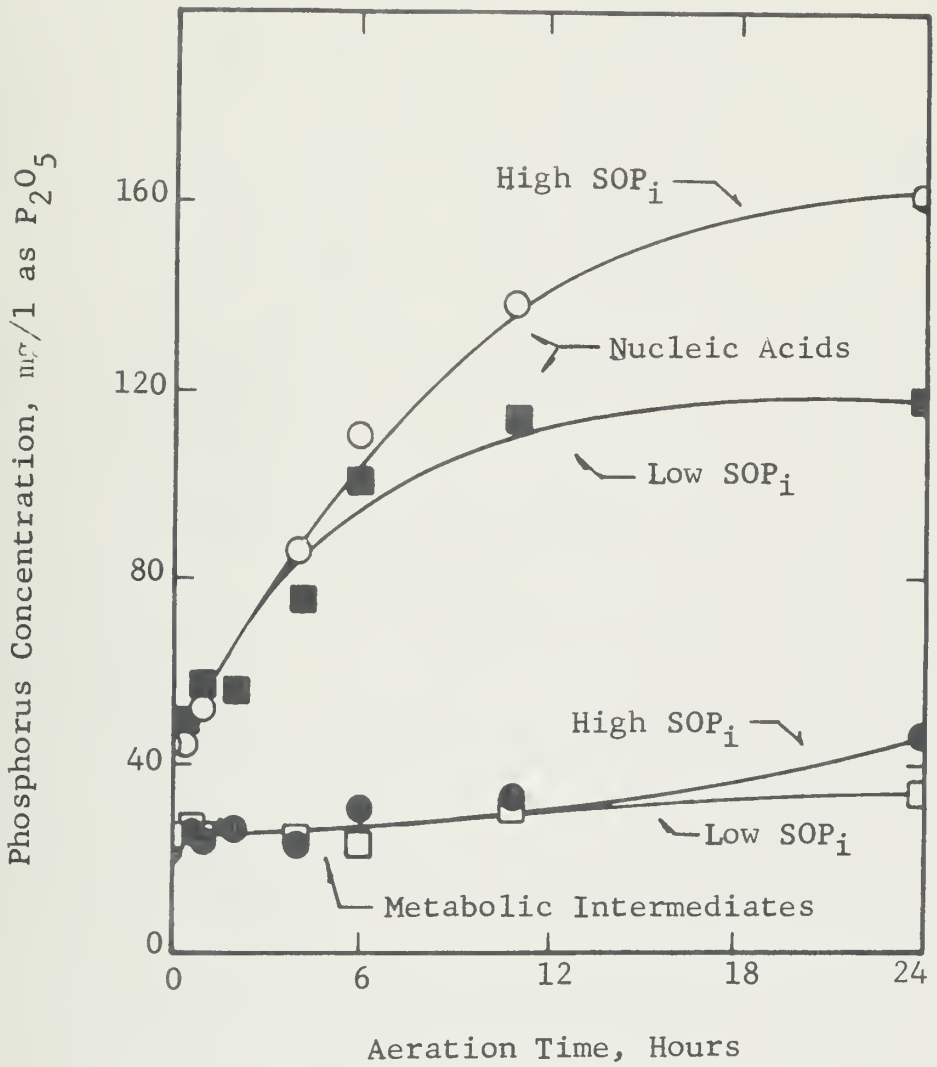

FIGURE 26 PHOSPHORUS CONCENTRATION OF NUCLEIC ACIDS AND METABOLIC INTERMEDIATES -- RESPONSE TO INITIAL SOLUBLE PHOSPHORUS CONCENTRATION.

TABLE 10

CELL FRACTIONATION STUDIES, DISTRIBUTION OF CELLULARLY ASSOCIATED PHOSPHORUS IN HIGH AND LOW SOP_i SYSTEMS

Time (min)	Phosphorus Content (Percent of Total Insoluble Phosphorus[a])											
	0.85% NaCl Wash		Metabolic Intermediates (Cold TCA)		Phospholipids (Hot ETOH)		Nucleic Acids (Hot TCA)		Protein (Residue)		Percent Recovery	
	High[b]	Low[c]	High	Low	High	Low	High	Low	High	Low	High	Low
3	1.2	0.8	36.2	28.9	3.8	3.2	77.0	62.0	5.0	3.3	123.2	98.2
30	1.1	0.8	42.6	32.4	6.8	3.7	73.0	60.0	4.7	5.2	128.2	102.1
60	3.7	1.5	30.8	30.1	5.7	4.7	64.6	65.2	4.1	5.8	108.9	107.3
120	1.3	0.5	26.6	--	9.2	7.0	56.0	54.1	2.9	4.4	96.0	--
246	1.0	1.3	17.8	18.2	4.4	6.1	57.0	58.0	3.6	3.4	93.8	87.0
360	0.8	0.3	21.9	15.7	2.5	2.7	77.5	69.0	3.7	3.9	106.4	91.6
660	0.5	0.2	19.3	19.6	2.9	2.6	79.0	71.6	3.3	3.6	105.0	97.6
1440	0.7	0.2	22.5	20.8	2.4	4.0	78.4	71.1	5.1	4.8	109.1	100.9
Mean	1.3	1.0	27.3	23.7	4.7	4.2	71.6	63.9	4.0	4.3	108.9	97.8

[a]Based on average total gross phosphorus.

[b]High = SOP_i of 179 mg/l as P_2O_5.

[c]Low = SOP_i of 90 mg/l as P_2O_5.

increase in MLSS concentration was essentially the same for both of these systems. Thus it was concluded that the only significant difference in the functioning of these two systems was the increase in soluble phosphorus uptake exhibited by the system containing the higher initial phosphorus concentration. It should be pointed out, however, that the increased uptake of soluble phosphorus in the higher SOP_i system probably resulted in a slightly higher MLSS concentration in this system. Such a slight increase, however, would be extremely difficult, if not impossible, to detect by the method that was used for the determination of MLSS.

In summary, all of the data obtained during this phase of the investigation offer a convincing argument that the biological uptake of soluble phosphorus by activated sludge systems is not, in all cases, limited by substrate availability or the synthesis of new cells. Under certain conditions, i.e., systems containing large amounts of soluble phosphorus relative to the amount of usable substrate, activated sludge systems appear to be able to take up soluble phosphorus in amounts much greater than those which would be predicted on the basis of usable substrate alone.

Thus it is possible to postulate that the biological uptake of phosphorus by activated sludge involves at least two stages or mechanisms. One of these mechanisms, which will here be termed the base mechanism, appears to be related to, and is probably controlled by, the availability of usable substrate. In other words, the cells have a basic need for phosphorus in the synthesis of new cells. This need is proportional to

the amount of cellular synthesis that occurs in the system, which in turn, is related to the availability of usable substrate. Thus, in a given system, a certain amount of usable substrate will be utilized for the synthesis of new cells, which in turn, will require that a certain amount of phosphorus be taken up by the system, to be used in the synthesis reactions. The second, or "luxury" mechanism, appears to be related directly to the system's soluble phosphorus concentration, and probably becomes operational only when the amount of soluble phosphorus present in the system exceeds that which is required for the functioning of the base mechanism. Thus, this "luxury" uptake may be envisioned as a storage mechanism, developed by the cells to insure that phosphorus is available for synthesis during periods when the soluble phosphorus content of the surrounding medium is low.

VI. ENGINEERING SIGNIFICANCE

The results obtained during this investigation contain several findings which are of practical engineering significance.

To begin with, it has been observed that, under certain conditions, the biological uptake of phosphorus by activated sludge systems can result in significant reductions in the soluble phosphorus content of biologically treatable wastewaters. For example, one of the experimental systems studied during this investigation was operated at an initial COD:P loading of about 17:1, which is probably a close approximation of the BOD:P ratio in today's domestic sewage.* It was observed that this system was able to biologically remove approximately 83 percent of the system's total soluble phosphorus during a 24 hour aeration period. Needless to say, percent removals of this order of magnitude are highly significant.

In addition, it should be recalled that these systems were maintained on a purely soluble substrate, so that the total uptake of soluble phosphorus in these systems was restricted to that amount which could be removed biologically. However, in the treatment of domestic and industrial wastewaters, this restriction might not apply. Thus it is entirely plausible that the activated sludge treatment of wastewaters containing significant amounts of colloidal and suspended material and/or multivalent cations, might be expected to effect even higher degrees of phosphorus removal, as a result of sorption or precipitation reactions involving these materials and phosphorus.

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*assuming an average BOD of 200 mg/l (58), and an average phosphorus concentration of 10 mg/l as P (16, 18, 65).

The results of this investigation also suggest that the detention times normally used in activated sludge aeration tanks may not be sufficient to effect optimum biological removal of phosphorus. This investigation showed that only about 50 percent of the biologically removable phosphorus was taken up during the first 4 to 6 hours of aeration. This seems to indicate that much longer aeration periods than those now being employed may be necessary for the effective biological removal of phosphorus by activated sludge. It was also observed that longer aeration periods, up to 24 hours, served to increase the total uptake of soluble phosphorus. The aerobic leakage of phosphorus from the sludge solids back into the aqueous phase as a result of prolonged aeration periods, which has been reported by other investigators (12, 14, 24), was not observed during these studies.

It was also observed that the optimum uptake of soluble phosphorus by activated sludge requires that the system be operated in a manner designed to insure that a dissolved oxygen concentration of approximately 2 mg/l is maintained throughout the aeration tank. This value, of course, is considerably greater than the value normally used in the design of activated sludge aeration facilities (58). Hence it would appear that the design and operation of aeration systems must be altered significantly before activated sludge systems can be effectively utilized for the removal of phosphorus.

These studies have also revealed that the system's MLSS concentration, at least within the range of 750 to 8000 mg/l, does not exert a significant influence on the uptake of soluble phosphorus by activated sludge. Therefore, presently accepted criterion of MLSS concentration,

based on the amount needed to affect satisfactory BOD removal, should also insure effective uptake of soluble phosphorus.

A similar situation is also apparent with regard to pH. Here it was learned that the optimum pH condition for the removal of soluble phosphorus by activated sludge was approximately 7.5 to 8, i.e., a slightly alkaline media. Since many wastes are naturally buffered within this range (58), pH adjustment to affect more effective removal of soluble phosphorus need not be considered, except in special cases.

Earlier investigators (12, 14, 22, 23, 26, 64) have reported that activated sludges may leak, or secrete, soluble phosphorus from the sludge solids back into the aqueous media when subjected to anaerobic conditions. This conclusion, coupled with the fact that anaerobic conditions often develop in secondary clarifiers, has led certain of these investigators (12, 14) to suggest that special and costly aerobic devices, such as froth flotation separators, may be required for solids separation where maximum soluble phosphorus removal is desired. However, several times throughout the course of this investigation, experimental activated sludges, rich in phosphorus, were subjected to anaerobic conditions, often for as long as 18 hours. An increase in the system's soluble phosphorus concentration resulting from such treatment was never observed. It was therefore concluded that with well oxidized sludges, such as those used in this investigation, this phenomenon does not occur to any significant degree. This conclusion is also supported by the data of Vacker et al. (29). This observation is quite significant from an engineering viewpoint, since it means that conventional clarification

units may be employed for solids separation, without a deleterious effect on phosphorus removal. Thus the use of special, costly, solids separation devices, in situations where maximum phosphorus removal is desired, may be avoided.

Perhaps the most significant conclusion to be derived from this investigation was the fact that activated sludge microorganisms do indeed take up significant amounts of phosphorus. This means that the biological solids produced in the activated sludge process contain considerable amounts of phosphorus. This fact is extremely important when considered in relation to one of the commonly used methods for disposing of these solids. In many waste treatment plants, these biological solids are disposed of by anaerobic digestion. In the digester, the solids undergo anaerobic decomposition, so that the organic matter is degraded to methane, carbon dioxide, and other inorganic compounds. As this degradation occurs, the phosphorus which was contained in the microbial cells is released, and, for the most part, remains in solution in the digester fluid. In many waste treatment plants the solid matter remaining is then separated from the digester liquid, and the liquid portion, heavily laden with soluble phosphorus, is returned to the aeration tank. Thus it is that, in many cases, the soluble phosphorus which the friendly organisms have worked so hard to remove, is eventually thrust back upon them again. It is hardly any wonder then, that the microorganisms, treated in such a hostile manner, refuse to remove very much of the phosphorus from the waste. It should be obvious from this discussion that the return of digester supernatant to the plant flow is, in relation to phosphorus removal, a self-defeating process.

VII. SUMMARY AND CONCLUSIONS

This investigation has shown that, given the proper conditions, a heterogenous population of microorganisms characteristic of activated sludge may be utilized to attain significant reductions in the soluble phosphorus content of biologically treatable wastewaters. The conditions necessary for this occurrence were shown to include the presence of an active microbial population, sufficient quantities of both organic and inorganic nutrients, an aerobic environment, and an adequate period of contact between the microorganisms and the waste being treated. It was also shown that essentially all of the phosphorus removed from solution under these conditions could be traced to the microbial cells composing the activated sludge. Approximately 60 percent of this phosphorus could be traced to the cells' nucleic acid fraction, and about 20 percent was found in the fraction containing metabolic intermediates and other low molecular weight phosphorus compounds.

The above observations lead to the conclusion that the uptake of soluble phosphorus in activated sludge mixed liquors is related to, and controlled by, the metabolic activity of the aerobic microorganisms present in the system.

Several other mechanisms which had been proposed as explanations for the observed disappearance of soluble phosphorus from activated sludge mixed liquors were examined experimentally. None of these mechanisms was found to be of significance in reducing the soluble phosphorus content of the mixed liquors used in this investigation.

A number of variables which were purported to affect the uptake of soluble phosphorus in activated sludge mixed liquors were studied. These included MLSS concentration, substrate concentration, dissolved oxygen concentration, degree of mixing, pH, and soluble phosphorus concentration. It was shown that, within the limits studied, substrate concentration, dissolved oxygen concentration, pH, and soluble phosphorus concentration exerted a pronounced influence on the rate and magnitude of soluble phosphorus uptake. On the other hand, MLSS concentration and degree of mixing exerted no observable effect on this uptake.

The secretion, or leakage, of soluble phosphorus from sludge solids back into solution, reported by other investigators to occur under anaerobic conditions, was never observed during this investigation. It was concluded that this secretion does not occur in well acclimated, well oxidized systems.

In view of the findings of this investigation, the following principal conclusions may be drawn:

1. The activated sludges used in these investigations were able to achieve significant reductions in the soluble phosphorus content of the mixed liquor during a 24 hour aeration period. For the systems operated with an initial COD:P ratio of approximately 75:1, about 90 percent of the systems' soluble phosphorus was taken up during this aeration period.

2. Only about 50 percent of the ultimate, i.e., 24 hour, uptake of soluble phosphorus occurred in the first 6 hours of aeration. This implies that activated sludge plants utilizing abbreviated aeration

periods may be rather limited in their phosphorus removal capability.

3. The primary cause of soluble phosphorus uptake in the activated sludge systems used for this investigation is the aerobic metabolic activity of the microorganisms composing the activated sludge. Essentially all of the phosphorus removed from solution during aeration of these units was traced to the cells composing the sludge. Only small amounts, approximately 1 percent, of the phosphorus taken up by the cells could be removed by washing with isotonic saline solution. The remainder of the phosphorus removed from solution was distributed in various fractions within the cell, principally in the form of (a) nucleic acids and (b) metabolic intermediates, and other low molecular weight phosphorus molecules. These two fractions were found to contain approximately 60 and 20 percent, respectively, of the cells' total phosphorus. Smaller amounts of this phosphorus were found in the cells' phospholipid and phosphoprotein fractions.

4. There was no discernable difference in either the rate or extent of soluble phosphorus uptake in five different activated sludge systems with MLSS concentration ranging from 750 to 8000 mg/l. This implies that MLSS concentration, at least within the range employed by most activated sludge plants, is not a factor in phosphorus removal.

5. In systems having a constant initial COD:P ratio of approximately 75:1, it was shown that the uptake of soluble phosphorus by activated sludge was proportional to the uptake of soluble COD. For these systems, this relationship may be expressed as follows:

Soluble Phosphorus Removal Rate = 0.0129 lbs P/(lb COD)(day)

The corresponding ratio of COD uptake to P uptake is approximately 79:1.

Studies on these same systems also revealed that the ratio between MLSS increase and phosphorus uptake was approximately 42:1, or, approximately 0.23 lb P/(lb MLSS increase)(day).

It was also found that the uptake of soluble phosphorus by the activated sludge used in this investigation is affected not only by COD uptake, but also by the system's initial soluble phosphorus concentration. Thus it is emphasized that the values given here apply only to the special case where the initial COD:P ratio was approximately 75:1.

6. The initial soluble phosphorus content of the mixed liquor exerts a marked effect on the uptake of soluble phosphorus by activated sludge. In identical systems operated at different initial soluble phosphorus concentrations the initial rate of uptake was the same, but the magnitude of the uptake was increased in the systems having higher initial concentrations of soluble phosphorus. It was concluded that under certain conditions, i.e., low initial COD:P ratios, soluble phosphorus may be taken up in amounts greater than that needed for normal cell growth.

7. Soluble phosphorus reduction in activated sludge mixed liquor is dependent upon the DO concentration of the mixed liquor. DO levels of approximately 2 mg/l appear to be necessary for the optimum uptake of soluble phosphorus.

8. Soluble phosphorus uptake in acclimated activated sludge systems was shown to be influenced by the pH of the system. Units operated at pH 6.5 did not take up soluble phosphorus as readily as those operated at pH 7.5. However, there was little further reduction in the

uptake capacity of systems operated at pH 6.0. On the other hand, systems operated at pH 9.0 appeared to be as effective in the removal of soluble phosphorus as those operated at pH 7.5. It was concluded that slightly alkaline systems were most efficient in removing soluble phosphorus.

VIII. SUGGESTIONS FOR FUTURE WORK

In view of the conclusions arrived at from the present study, it is suggested that this investigation should be extended to include the following:

1. Similar studies, employing other than purely soluble substrates, to determine the influence of colloidal and suspended organic matter on the uptake of phosphorus by the activated sludge process.

2. An investigation of the effect of temperature on the uptake of soluble phosphorus by activated sludge mixed liquor.

3. Studies with lower initial COD:P ratios to determine if the phosphorus uptake mechanism in activated sludge can be saturated.

4. Studies to determine if the relationships observed in these batch studies can be duplicated in continuous flow systems.

5. Investigations to establish the role of cellular energy storage products in the uptake of soluble phosphorus by activated sludge.

6. Studies to determine if the uptake of soluble phosphorus by activated sludge microorganisms is related to their uptake of dissolved oxygen. If such a relationship could be demonstrated, it would offer further proof of the biological nature of phosphorus uptake by activated sludge.

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APPENDIX A

Reagents used in the determination of total phosphate (39).

i. Perchloric acid - 70-72 percent, ACS reagent grade. This may be purchased at this strength.

ii. Nitric acid - concentrated, ACS reagent grade.

iii. 5 percent ammonium molybdate solution - dissolve 5 gm ammonium molybdate, ACS reagent grade, in 100 ml distilled water.

iv. Elon reducing agent - dissolve 1.25 gm P-methylaminophenol sulfate (also known as elon, graphol, or metol), 31.25 gm sodium bisulfite, and 6.0 gm sodium sulfite, all ACS reagent grade, in 250 ml distilled water. Store solution in dark bottle in refrigerator.

VITA

Millard Wayne Hall was born at Edison, Georgia, on May 30, 1936. After graduating from Griffin High School, in Griffin, Georgia, he enrolled in the Vanderbilt University College of Engineering. He left Vanderbilt University temporarily in March, 1956, and in July of that year entered the U. S. Army Corps of Engineers. After serving in Germany, he was honorably discharged in 1959, and returned to Vanderbilt University to pursue studies in Civil Engineering. He was graduated from Vanderbilt University in June 1962, with a B. E. in Civil Engineering.

In September 1962, he entered the Graduate College of the University of Illinois, as a recipient of a U. S. Public Health Service Traineeship award. He received an M. S. in Sanitary Engineering from the University of Illinois in October 1963. As part of the requirements for his Master's degree, he prepared a report of a Special Problem entitled, "Inorganic Compounds If Phosphorus in the Activated Sludge Process."

From September 1963 to the present he has been a doctoral candidate in the Sanitary Engineering program at the University of Illinois. During the first three years of this period he received financial support from a U. S. Public Health Service Research Fellowship, and from the Ford Foundation Load Program.

In September 1966, he was appointed as Instructor in the Department of Civil Engineering, University of Illinois. In January of 1967, he resigned this position to become Assistant Professor of Civil Engineering at the University of Maine, where he is presently located.

He is married to the former Miss Marlene Murphy of Griffin, Georgia, and they have two children, Crystal Dianne, age nine, and Michael Wayne, age 4.

He is a member of Phi Kappa Sigma social fraternity, and the Fellowship of Christian Athletes. He is also an ordained Deacon in the Southern Baptist Church.

His professional associations include the American Water Works Association, the Water Pollution Control Federation, the American Society of Civil Engineers, and the Society of Sigma Xi.

His professional publications are listed below:

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